EVALUATION OF SALIVARY BIOMARKER –LACTATE DEHYDROGENASE IN TOBACCO USERS, ORAL LEUKOPLAKIA AND ORAL SQUAMOUS CELL CARCINOMA- A COMPARATIVE STUDY.

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This is to certify that the dissertation titled **"EVALUATION OF SALIVARY BIOMARKER –LACTATE DEHYDROGENASE IN TOBACCO USERS, ORAL LEUKOPLAKIA AND ORAL SQUAMOUS CELL CARCINOMA- A COMPARATIVE STUDY"** is a bonafide work done by **Dr. EDWINA. J,** Postgraduate student, during the course of the study for the degree of **MASTER OF DENTAL SURGERY** in the speciality of **ORAL MEDICINE AND RADIOLOGY**, Vivekanandha Dental College for Women, Tiruchengode, during the period of 2015-2018.

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TITLE OF DISSERTATION	Evaluation Of Salivary Biomarker –Lactate Dehydrogenase in Tobacco Users, Oral Leukoplakia and Oral Squamous Cell Carcinoma- A Comparative Study
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Dr.Edwina.J

LIST OF ABBREVIATIONS USED

OC	Oral Cancer
WHO	World Health Organization
SCC	Squamous Cell Carcinoma
OSCC	Oral Squamous Cell Carcinoma
ICD	International Classification of Disease
OPMD	Oral Premalignant Disorder
OL	Oral Leukoplakia
OSMF	Oral Submucous Fibrosis
LDH	Lactate Dehydrogenase
IU/L	International Units Per Litre
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
ANOVA	Analysis of Variance

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INTRODUCTION

Oral cancer (OC) was recognised in India as early as the 600 BC. The Sushruta Samhita, treatise on Indian surgery written describes the conditions granthi and arbuda as malignant conditions affecting the body. Today, the World Health Organization (WHO) considers oral cancer as one of the greatest public health challenge in India.[1]

OC are considered the sixth most common type of cancer in the world. The Annual global incidences according to Warnakulasuriya S, amounts to around 275,000 cases of oral and 130,300 cases of pharyngeal cancers excluding cancers of the nasopharynx. Among which two third of the cases occur in developing countries. The Indian subcontinent has long been regarded as the global epicentre of oral cancer.[2]

All over the world over 90% of malignancies affecting the oral cavity and maxillofacial region are diagnosed as squamous cell carcinomas (SCC).[3] According to Willis in 1952, neoplasm can be defined as an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after cessation of the stimuli which evoked the change.[4] Malignant neoplasms of the lip, oral cavity and oropharynx [International Classification of Diseases (ICD)10 codes: C00–C14], excluding other pharyngeal sites (C11–C13), are often grouped together as oral cancer.[5]

In high risk countries such as Bangladesh, India, Pakistan, and Sri Lanka oral cancer is most common and accounts for about a third of all cancers. More than 100,000 new cases occur every year in south and south-east Asia, with poor prospects of survival. [3] Nowadays, about 90–95% of all oral cancer cases in the world, including that in India, are squamous cell carcinomas (SCC) arising from lining mucosa of the oral cavity.[1] The International Agency for Research on Cancer GLOBOCAN project has stated that

India's cancer incidence may double in the coming 20 years, from over a million new cases present in 2012 to greater than 1·7million cases by the year 2035. These predictions show that the number of deaths due to cancer will also rise from about 680 000 to 1·2 million during the same time.[6]

In the Indian context, oral cancer etiology is dominated by tobacco use, especially because of smokeless tobacco, areca [betel] nut consumption and alcohol abuse, all of which frequently act in the presence of poor diet and poor dental health.[7] The similar scenario can be seen in areas of central Asia and the rest of the Indian subcontinent due to increased usage of betel quid, with or without smokeless tobacco, smoking, alcohol consumption, and poor diet. [8]

Tobacco smoking carries a six-fold risk of developing oral cancer compared to not smoking. Oral cancer is also six times more likely to develop in alcohol drinkers than in non-drinkers. [9] The combination of tobacco and alcohol use poses a twenty four fold increased risk of oral cancer for users compared to non-users. Though tobacco and alcohol use are traditionally the greatest risk factors, it is important to consider other known risk factors, such as betel quid chewing, in certain ethnic populations. Betel quid chewing is popular in Indian and Taiwanese populations and is associated with a significantly increased risk of oral cancer.[1] Areca nut, narcotics and cannabis use has also been found to be a risk factor for oral cancer.[8]

Several oral premalignant disorders (OPMDs) precede the development of OSCC. One of the most commonly encountered lesion is leukoplakia. The global prevalence of oral leukoplakia has been estimated at 2%.[10] Oral leukoplakia can be of homogeneous and non-homogeneous variants. It may start as a homogeneous type but over time develop a verrucous appearance containing various degrees of dysplasia. The malignant

potential is very high, and verrucous carcinoma or squamous cell carcinoma may also be present at the primary examination.[11]

Development of diagnostic aids will lead to increasingly sensitive and specific detection of pre-malignancy and malignancy states. Potential biomarkers are necessary to observe the progress of OPMD to OC. These are required for the effective prevention and therapeutic strategies. This will eventually result in alleviation of the public health burden imposed by oral cancer.

Saliva as a bio-fluid has been extensively studied biochemically and physiologically. Salivary biomarkers play an important role in non-invasive detection of various diseases. Biomarkers are cellular, biochemical, molecular, or genetic alterations by which a normal, abnormal, or simply biologic process can be recognized or monitored. Salivary biomarkers can also be used to detect premalignant disorders and oral cancer.[12]

Lactate dehydrogenase (LDH) is a cytoplasmatic enzyme present in essentially all major organ systems. The extracellular appearance of LDH is used to detect cell damage or cell death. Salivary LDH levels have been rarely studied in OC and OPMDs. The study was done to evaluate the LDH levels in oral leukoplakia and oral cancer using the relatively non-invasive saliva as the diagnostic tool.[13]

AIMS AND OBJECTIVES

AIM:

The aim of the present study was to evaluate and compare the salivary biomarker lactate dehydrogenase in tobacco users, oral leukoplakia, oral squamous cell carcinoma and healthy controls.

OBJECTIVES:

The objective of the present study was to find out the significance of lactate dehydrogenase in salivary samples of tobacco users, oral leukoplakia, oral squamous cell carcinoma and healthy controls.

To ensure whether estimation of these markers in oral leukoplakia and oral squamous cell carcinoma is valuable in assessing the malignant risk potential.

REVIEW OF LITERATURE

The worldwide estimate for oral cancer accounts for 2%–4% of all cancer cases.[14] Cancer is considered the second most common disease in India. It was said that more than five people die from OC every hour each day in India.[1]

Epidemiology of oral cancer

Figure 1 shows the estimated incidences and mortality in men and women of all ages in India. Approximately 70,000 new cases and more than 48,000 oral cancer-related deaths occur yearly. In most regions of India, oral cancer is the second most common malignancy diagnosed in men, accounting for up to 20% of cancers, and the fourth most common in women.[15]

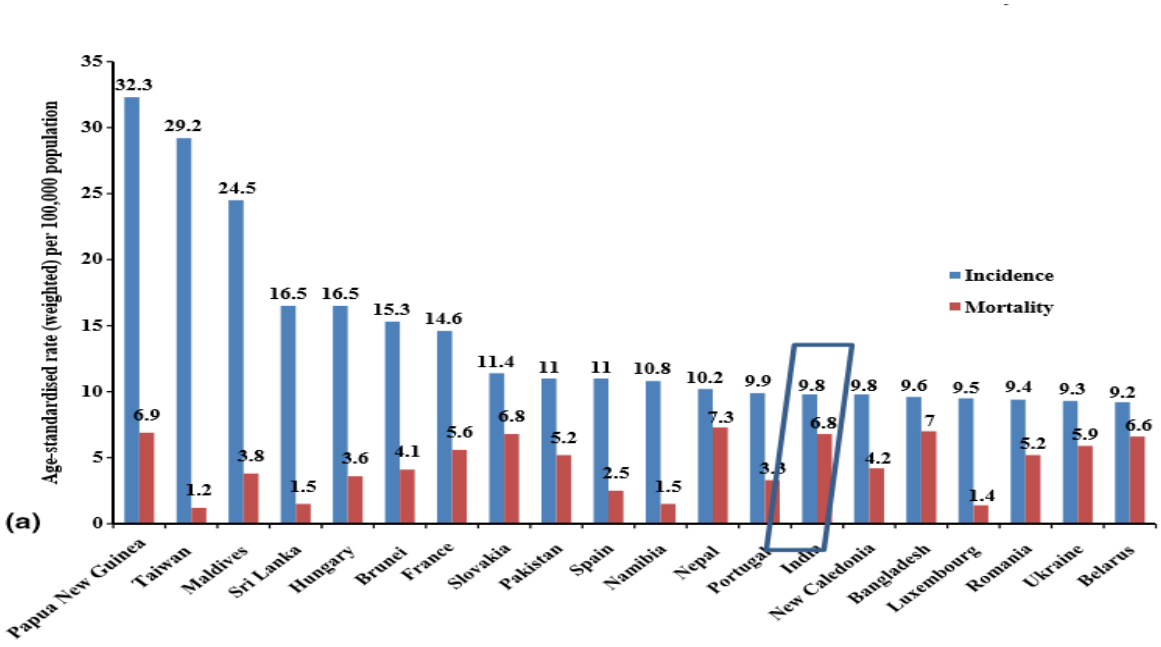


Figure 1 - (a) Incidence and mortality of the lip and oral cavity cancers among males of all ages in the 20 countries with the highest rates in 2008. India ranks 14th in incidence.

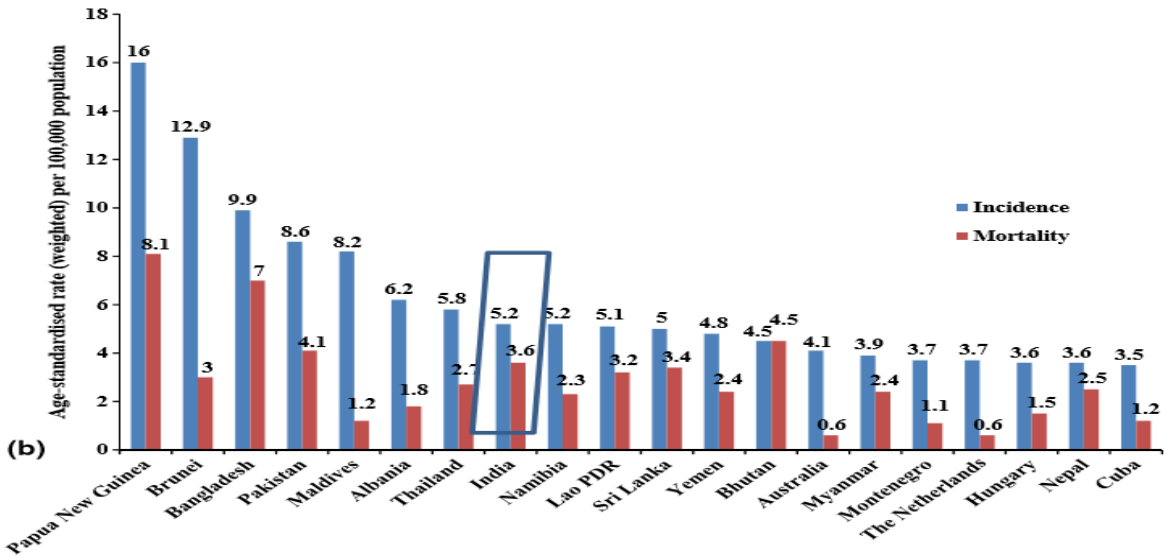


Figure 2 - Incidence and mortality rates of cancers of the lip and oral cavity among females of all ages in the 20 countries with the highest rates in 2008. India ranks eighth in incidence and fifth for mortality. Source: GLOBOCAN 2008.

Swaminathan. R *et al*. stated that the average number of cases incident in the city of Chennai during the year 2002-06 includes 124 cases of mouth cancer and 119 cases of tongue cancer as recorded in the Chennai Cancer Registry.[16]

Tobacco

Tobacco usage is a known risk factors for oral cancer. Tobacco contains numerous potent carcinogens, including polycyclic aromatic hydrocarbons, nitrosamines, nitrosodiethanolamine, polonium and nitrosoproline. Tobacco smoke contains carbon monoxide, hydrogen cyanide, thiocyanate, nicotine, and metabolites of these constituents. Nicotine is a strong addicting drug. Several epidemiologic studies have stated that up to

80% of OC patients were smokers. Continuing smoking after cancer treatment has the risk of developing several primary cancers, recurrent and second primary oral cancers.[11]

Oral leukoplakia

Oral leukoplakia is defined by the WHO as "a white patch or plaque that cannot be characterized clinically or pathologically as any other disease." Oral cancer is often preceded by precancerous lesions and conditions such as leukoplakia, erythroplakia and oral submucous fibrosis. [17] A precancerous lesion can be considered as a benign, morphologically altered tissue that has a greater than normal risk of malignant transformation.[18]

Oral leukoplakia is the most commonly encountered oral precancerous lesion with the malignant transformation ranging from 1.58 to 27.27%. The occurrence of dysplastic changes are not certain and it can transform from hyperkeratosis to carcinoma without any recognizable dysplasia. [17]

Tumour markers

There are several advancements being made for the early detection of oral malignant and premalignant lesions. Biomarkers are being developed extensively to accurately diagnose the cancerous and precancerous conditions. Biomarkers are measurable and quantifiable biologic parameters that may serve as indicators for health and physiology-related assessments, such as pathogenic processes, environmental exposure, disease diagnosis and prognosis or pharmacologic responses to a therapeutic intervention.[19]

There are several circulatory tumour markers which can be detected in the serum but saliva is a unique body fluid that can be easily obtained. Salivary diagnostics is now widely used for the development of molecular diagnostics as it contains not only

components found in serum but also offers several advantages over serum. Saliva collection is cost-effective, safe, easy and non-invasive.

Salivary diagnostics

In the recent years, many non-invasive diagnostic techniques have gained popularity for the early detection of oral cancer. Saliva has recently gained immense popularity as a diagnostic fluid, as several biomarkers are considerably elevated in saliva. [20]

A total of 1166 salivary proteins were identified out of which two third of them appeared exclusively in saliva and this is known as the salivary proteome. [21] In the future, salivary tests can be undertaken to provide an easy practical, convenient, and comfortable test for the early diagnosis of various malignancy states.

Lactate dehydrogenase enzyme

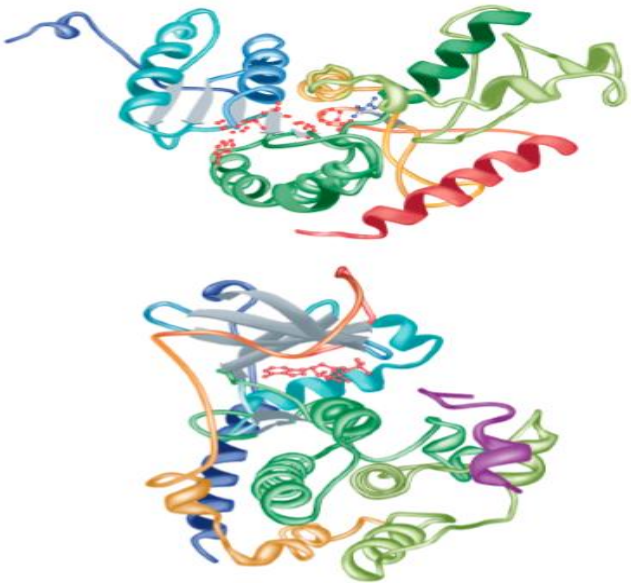


Figure 3 LDH enzyme ribbon diagram. Shown is the three dimensional structure of lactate dehydrogenase with the substrates NADH (red) and pyruvate (blue) bound.[22]

Lactate dehydrogenase enzyme belongs to a group of oxidoreductases enzymes. LDH has two terminal domains, an N-terminal for NAD+-binding domain and a C-terminal for binding to the second substrate, pyruvate they utilize NAD(P)+/NAD(P)H for the oxidation and reduction of a wide range of metabolites. Lactate dehydrogenase (LDH) is a tetrameric enzyme consisting of two monomer types: H (for heart) and M (for muscle) that combine to yield five LDH isozymes: HHHH (I_1), HHHM (I_2), HHMM (I_3), HMMM (I_4), and MMMM.[15] Tissue-specific expression of the H and M genes determines the relative proportions of each subunit in different tissues. Isozyme I_1 predominates in heart tissue, and isozyme I_5 in liver. Thus, tissue injury releases a characteristic pattern of LDH isozymes that can be separated by electrophoresis and detected using a coupled assay. LDH is widely used for diagnostic enzymology. The level of LDH in serum has been used as biomarkers in the fields of oncology, cardiology, hepatology and hematology for diagnosing and monitoring of treatment progress in the malignancy states like non-Hodgkin's lymphoma and ovarian dysgerminoma.[23]

Beena P. Patel, Upendra M. Rawal, Rakesh M. Rawal, Shilin N. Shukla and Prabhudas S. Patel in 2008 studied about the effect of tobacco on antioxidative enzymes in Oral Cancer. In this study fifty healthy controls and one hundred forty oral cancer patients were included. and, classified as “habitual controls” and “nonhabitual controls” having tobacco habits and no tobacco habits, respectively, were included in the study. Tissue samples were collected from normal and malignant locations. The tissue levels of antioxidant enzymes were assayed by spectrophotometric methods and concluded that the risk of malignancy is increased in habitual controls with decreased antioxidant enzymes and increased lifetime tobacco exposure.[24]

STUDIES CONDUCTED ON LACTATE DEHYDROGENASE ENZYME.

Pereira T, Shetty S, Pereira S had estimated the serum lactate dehydrogenase level in patients with oral premalignant lesions/ conditions and oral squamous cell carcinoma. This study was carried out in a total of 110 subjects, out of which 55 subjects had premalignant disorders, 30 were diagnosed with OSCC and 20 were of healthy controls. The serum LDH levels were assessed for the three groups. Student's T test was used to analyse the parameters in the three group. The mean LDH in serum was estimated to be 339.90 IU/L for the control group, 488.67 IU/L for leukoplakia, 743.30 IU/L for well differentiated OSCC and 988.50 IU/L for moderately differentiated OSCC. There was a progressive rise in the serum LDH level from control group to OSCC group. The results were statistically significant. It was concluded that since LDH is an intracellular enzyme involved in anaerobic glycolysis. The appearance of LDH in serum is used to detect cellular damage or cell death. Long term carcinogenic changes may interfere with glycolysis during carbohydrate metabolism. Malignant tumour tissue have increased mitotic index and they produce more lactic acid due to breakdown of glycoprotein. This causes an increase in circulatory LDH levels in serum. Hence, LDH level can serve as a biochemical tool in assessing the malignant potential of premalignant lesions and be used to screen the cases of oral malignancy as an adjunct to diagnosis.[25]

Patel S, Metgud R had researched on the level of salivary lactate dehydrogenase in oral leukoplakia and oral squamous cell carcinoma. In his study, subjects were grouped into three categories comprising of healthy controls, OL and OSCC. Unstimulated whole saliva was collected and the salivary LDH was estimated using a semi-automatic biochemical analyser. The data was analysed using student t- test for the three groups. Also, ANOVA test was used to test the significance of difference between the histopathological grades of OSCC. The salivary LDH values for the control group

was 261.16±75.851IU/L, for the leukoplakia group was 497.00±100.404 IU/L and for the OSCC group was 686.40±81.752 with statistically significant P value. The intragroup levels of salivary LDH in the OSCC group was estimated as 745.53±98.403 IU/L for well differentiated OSCC, 799.129±89.404 for moderately differentiated OSCC and 828.25±79.752 for poorly differentiated OSCC with a statistically insignificant P value. It was postulated that LDH is a cytoplasmatic enzyme present in most of the cells. Increased LDH activity can occur during genomic cellular changes in premalignant and malignant states. Increased LDH in the whole saliva may be due to direct diffusion of fluids from the oral epithelium and the gingiva. The development of OSCC corresponds to high glycolytic activity with a direct increase in the production of LDH. Therefore, it was concluded that estimating LDH can be a simple and an effective tool for early diagnosis of oral premalignant and malignant states. [26]

A study was conducted by **Gorogh T *et al*** to evaluate the LDH isoenzyme in OSCC. Five subjects who were histologically diagnosed with OSCC and prior to treatment were included in the study. The biopsy specimen was obtained from healthy and malignant sites and tissue was cultured and processed. The epithelial cell culture was obtained and isoelectric focussing was done on ultrathin layer gels. Quantitative and qualitative LDH isoenzyme patterns was determined using gel electrophoresis. The malignant cell lines showed 30% higher total LDH isoenzyme activity. It was also found that LDH-6 isoenzyme was present in all of the tumour cells but not in the normal cells. The isoenzyme LDH-7 and LDH-9 were present in relatively higher proportions in the tumour cell lines. This difference in LDH isoenzyme pattern was attributed to the fact that increasingly proliferating malignant cell population derive their energy from anaerobic glycolytic pathway, with a greater requirement for LDH to regenerate NAD

from NADH to support and sustain glycolysis. Hence LDH isoenzyme evaluation can help assessing the disease activity in oral malignancy. [27]

A research was done by **Rathore A, Nagarajappa AK, Sreedevi** on the serum levels of lactate dehydrogenase in oral carcinoma, leukoplakia and oral submucous fibrosis. The study was performed on 120 subjects who grouped into 4 groups. 30 subjects in each group of OSCC, OL, oral submucous fibrosis and healthy controls. The subjects in the study groups were clinically and histopathologically confirmed with the malignancy and premalignancy conditions. The serum was collected from the subjects and biochemical evaluation was done using semi-automatic analyser. Statistical analysis was performed. ANOVA test was done to compare the mean values between the groups. The mean serum LDH level in control group was 161.39 ± 36.14 IU/L, in the OSCC group was 323.83 ± 46.80 IU/L, OL group was 277.91 ± 33.34 IU/L and OSMF group subjects was 249.68 ± 44.65. This showed a progressive increase in the mean serum LDH activity from OSMF group to OL group to OSCC group with a statistically significant P value of < 0.05. In this study males were more commonly affected than females in the study groups. The increased serum LDH may be due to the fact neoplastic cells showed morphologic and enzymatic changes this may lead to increased tissue destruction or incomplete cellular destruction, causing increased circulatory LDH. In this study it was concluded that serum LDH can be used as a potential biomarker to diagnose malignant and pre-malignant disorders.[28]

A study was performed by **Joshi PS and Golgire S**, on salivary LDH isoenzyme pattern in squamous cell carcinoma and oral leukoplakia. In this study 30 samples in each group of clinically and histopathologically diagnosed OL and OSCC were included and 30 healthy subjects were taken as control group. The whole saliva was collected and centrifuged. The resulted supernatant was used for biochemical evaluation using gel

electrophoresis method and LDH isoenzyme activity was determined. The biopsy specimen of OL and OSCC cases were histopathologically graded according to dysplasia and differentiation respectively. The total salivary LDH was estimated to be 267.2 IU/L in the control group, 519.3667 in the OL group and 788.7333in the OSCC group. In the OL group there was significant increase in the isoenzyme activity of LDH 2, LDH 3, LDH 4 and LDH 5 when compared with control group. Also in a progressive increase in the isoenzymes LDH 5, LDH 4, LDH 3 and LDH 2 was observed with increasing grades of dysplasia. In the OSCC group, there was a significant increase in levels of LDH 3, LDH 4 and LDH 5 when compared to control group. The intragroup observation in the OSCC showed that there was a progressively significant increase in isoenzyme LDH 5 in well, moderate and poorly differentiated cases. It was concluded that there is an overall increased salivary LDH isoenzyme level in OL and OSCC cases and a significant correlation between levels of salivary LDH isoenzymes and histopathologic grades of dysplasia in OL and OSCC was seen. Salivary analysis of LDH will definitely provide the clinician and/ or the patient himself with an efficient, non - invasive and friendly new tool for diagnosis and monitoring of oral precancer and cancer.[29]

A research was conducted by **Dhivyalakshmi M and Maheswari T. N. U** to understand the significance of salivary LDH levels in OL and OSCC. The study included a total of 42 subjects, who were divided into three groups, healthy subjects formed the first group, clinically diagnosed cases of oral leukoplakia formed the second group and the third group consisted of subjects who were clinically and histopathologically diagnosed with OSCC. Unstimulated saliva was collected from all the patients and after centrifugation, the supernatant was subjected to biochemical analysis using auto analyser. Statistical analysis was performed using one way ANOVA test. The mean value of LDH was estimated to be 79.70 IU/L for control, 102.54 IU/L

for OL and 268.57 IU/L for OSCC. There was significant difference between the three groups with a p value of <0.001 for salivary LDH. Hence, the use of saliva for the evaluation of biomarkers for early detection of malignant risk potential may be more appropriate for oral cancer, as saliva reflects the health of oral tissues as it bathes the entire oral mucosa. It was found that salivary LDH could be a sensitive biomarker for the detection of OL and also aids in early detection of OSCC.[30]

Audrey M D'Cruz and Varsha Pathiyil had conducted a case-control study to compare the levels of salivary LDH in various pathological differentiation of OSCC patients. In this study 30 patients with OSCC and 30 healthy controls were included. The OSCC group was divided further based on histopathological grading into well differentiated, moderately differentiated and poorly differentiated. Unstimulated saliva was collected from all the patients and biochemical analysis was performed. The mean salivary LDH levels was 117.33 ± 19.37 IU/L in the control group , 355.83 ± 16.73 IU/L in the well-differentiated OSCC group, 484.18 ± 25.84 IU/L in the moderately differentiated OSCC group, and 620.35 ± 18.69 IU/L in the poorly differentiated OSCC group. There was a statistically significant difference between the three groups. It was analysed that the source of salivary LDH was largely non glandular and was due to the direct diffusion of the enzyme from the epithelial cells. Therefore, salivary LDH can be considered as an important biomarker for detection of OSCC.[31]

In a research done by **Balwant Rai, Rajnish Jain, S.C. Anand and Simmi Kharb**, the isoenzyme levels of salivary LDH was evaluated to assess its usefulness for screening of oral lichen planus.in this study a total of 35 subjects were included, 10 subjects who were diagnosed with oral lichen planus belonged to the study group and 25 subjects were included in the control group. Unstimulated whole saliva was collected

from all the participants. The LDH isoenzyme LDH-1, LDH -2, LDH-3, LDH-4, LDH-5, the H-subunit and the M-subunit of the enzyme were evaluated. Statistical analysis was performed using SPSS software and student t- test was done to compare the means. The results showed that the isoenzyme LDH-3, LDH-4, LDH-5 and M subunit of the enzyme were increased in patients with oral lichen planus with a statistically significant difference when compared to the control group. It was concluded that LDH isoenzyme LDH-1 to LDH-5 can be detected in the saliva and the elevated levels of the isoenzyme LDH-3, LDH-4, LDH-5 shows that salivary LDH can be a suitable biomarker for screening oral lichen planus.[32]

In a study conducted by **Shishir Ram Shetty *et al***, the salivary LDH levels were estimated in oral leukoplakia and OSCC. In this study they had recruited a total of 75 patients. 25 patients in each group of OL, OSCC and healthy controls. Unstimulated whole saliva was collected, process and Salivary LDH was estimated using biochemical analyser. Statistical analysis was carried out and one way ANOVA test was used to compare the data between the groups. The mean salivary LDH was 79.50 ± 4.67 IU/L for healthy controls, 136.46 ± 3.36 IU/L for OL and 148.77 ± 4.83 IU/L for OSCC. They have suggested that the salivary LDH levels were comparatively higher in OL and OSCC as LDH was considered as a ubiquitous enzyme that is elevated during pathological processes. Hence, salivary LDH can be considered as a crucial biomarker for OL and OSCC. [33]

A study by **Kavya Shree Lokesh, Jayanthi Kannabiran, Mahesh Dathu Rao** was undertaken to assess the salivary LDH levels in OSCC and to evaluate its potential as a biomarker for the diagnosis of OC. The study consisted of 30 patients diagnosed with OSCC and were divided into groups based on their histopathological differentiation

and 20 healthy patients belonged to the control group. Unstimulated whole saliva was collected from all the patients and processed. The enzyme activity was analysed using auto analyser. The results obtained showed that controls had a mean salivary LDH value of 497.00IU/L whereas the OSCC cases showed a mean value of 1225.40IU/L. Based on the pathological differentiation, the salivary LDH levels for the various grades of OSCC were compared and the mean values were 1049.07 IU/L, 1309.50IU/L and 1586.20IU/L respectively for well differentiated, moderately differentiated and poorly differentiated carcinoma. Thus it was concluded that the metabolic alterations in saliva can serve as an epidemiological marker of oral and oropharyngeal carcinogenesis by using salivary LDH. [34]

A research was carried out by **Langvad E, Zachariah J, Pindborg JJ**, on LDH isoenzyme pattern in oral leukoplakia, oral submucous fibrosis and OSCC in the Indian Subcontinent. The study was done on the biopsied oral mucous membrane specimens of OL, oral submucous fibrosis and OSCC. The sample included 135 biopsy specimens obtained from 108 subjects and control specimens included 32 subjects with clinically normal mucosa. The tissues were processes and enzymatic study was done using disc electrophoresis. The isoenzyme ratio LDH IV; LDH II was calculated. The mean ratio was calculated as 3.42 in patients without habits, 2.68 in patients with tobacco habits, 3.34 in OL cases, 2.54 in OSCC and 2.18 in OSMF cases. It was found that there was an absence of significant difference between the isoenzyme ratio (LDH IV/ LDH II) in OL, OSCC and OSMF cases. This can be attributed to the fact that there might be an increased LDH isoenzyme ratio in normal mucosa in the Indian subcontinent population due to the lifestyle patterns and prevalence of high tobacco usage.[35]

A study was done by **Sivaramakrishnan M, Sivapathasundharam B, Jananni M** to evaluate the serum and salivary LDH on healthy controls and oral submucous fibrosis patients. The samples consisted of 60 subjects who were divided into 2 groups. Group A consisted of 30 subjects who were diagnosed with oral submucous fibrosis (OSMF) and Group B consisted of healthy controls. Blood samples and unstimulated salivary samples were collected was used for the analysis. LDH estimation was done using enzyme assay kit and UV visible spectrophotometer. Statistical analysis was done and student t-test was used to compare the parameters between the two groups. The mean serum LDH level was 521.00 ±27.300IU for group A and 289.43±26.865 for group B. The mean salivary LDH value for Group A was 606.83 ± 60.09 U/l and for Group B was 80.73 ±20.06 U/l. The intergroup comparison results were statistically significant. The intragroup serum and salivary LDH in Group A was compared with the clinical staging of OSMF and it showed statistically insignificant results. It was proposed that malignant transformation from OSMF to OSCC involves alterations in the cellular glycolytic pathways that can change the LDH enzyme levels. LDH is said to be an oxidoreductase enzyme that catalyzes the conversion of pyruvate to lactate and vice versa. It was concluded that the salivary LDH considerably increased in the OSMF group. This study therefore proves that salivary LDH can play an important role in the early diagnosis and prognosis of oral submucous fibrosis. [36]

Abinaya Chari, Rajesh P, Prabhu S studied about the serum levels of LDH on smokeless tobacco consumers. In this study 3 groups were present. 35 patients with oral lesions who chewed tobacco were selected for the study group, the second group consisted of 10 patients with the habit of chewing tobacco and without any oral lesions. Ten healthy patients without the habit were included as controls. Serum LDH levels were assessed biochemically. The results showed that the average serum LDH in

patients with habit and lesion was 446.8 U/L; for patients with habit but without oral lesion serum LDH was 421.2 U/L, and for patients without a habit or lesion the serum LDH was 269.4 U/L. This showed that subjects with the habit of using smokeless tobacco and with lesions had greater serum LDH values when compared to the non-lesion group of subjects who had no tobacco chewing habits. [37]

In a research performed by **Rosalind Tayler, V. H. Cumberland, and D. W. Piper** on the colorectal mucosa obtained from subjects with colorectal carcinoma. It was discovered that there was a considerable difference in pattern of LDH isoenzymes in the mucosa which was proximal and distal to the carcinoma and was involved in malignancy showed similar resemblance to the carcinoma tissue. This differed from that of normal tissue. In the case of the uninvolved mucosa proximal to the carcinoma there was no difference in the LDH isoenzyme pattern and it was similar to the normal mucosa. Hence tissue LDH isoenzyme pattern can be a valuable tool to evaluate mucosal malignant changes. [38]

Henk J. Huijgen *et al* reviewed about the clinical importance for estimation of lactate dehydrogenase in serum which was applicable in the fields of oncology, cardiology, hematology and hepatology. It was said that LDH can be considered as a suitable tumour marker and can be useful for monitoring the disease progress and was relevant in determining the survival duration in Hodgkin's and non-Hodgkin's lymphoma. [23]

M. Drent, N.A.M. Cobben, R.F. Henderson, E.F.M. Wouters and M. van Dieijen-Visser reviewed the usefulness in monitoring the level of LDH as indicators of lung pathologies, like cell damage and inflammation. The presence of cytoplasmic cellular enzymes like LDH, in the extracellular space, may serve as indicators of cellular

disturbances caused by pathological conditions. Since LDH is considered an enzyme that is present in all the major organ systems, serum LDH level is abnormal in many disorders. The LDH levels in many tissues are considerably high when compared to its level in serum. For example, in heart it is 25,000U/g, in kidney it is about 15,000U/g; in liver it is 9,000 U/g; in skeletal muscle it is 9,000U/g and in lung it is around 9,500U/g. Thus, the tissue levels of LDH enzyme are about 500 times higher than the amount of LDH usually found in serum, and escape of the enzyme from a small amount of damaged tissue can increase the serum LDH level to a great extent. Though the LDH level is high in many tissues, they show a different isoenzyme pattern. In cardiac muscle, kidney and erythrocytes the isoenzymes LDH1 and LDH-2 are more predominate. Whereas in liver and skeletal muscle, the isoenzyme LDH-4 and LDH-5 are predominate. It was concluded that the measurement of LDH levels in pleural effusion and broncho-alveolar lavage fluid may provide information about injury to the lung and pulmonary endothelial cells.[39]

Khalida S. Merza, Sanad B. Alaaraji, Bashar H. Abdullah studied about LDH levels in serum and saliva of acute leukemia and oral squamous cell carcinoma patients. In the study saliva and blood samples were collected from subjects diagnosed with acute leukemia and OSCC. The LDH activity in the salivary and serum samples were biochemically analysed. The serum LDH level was estimated to be 634.2 ± 406.18U/L in acute leukemia, 311.2 ± 86.1U/L in OSCC, whereas in controls it was 229.5 ± 57.4U/L. the salivary LDH level was 319.2 ± 126.2U/L in acute leukemia, 325.4 ± 156.5U/L in OSCC and 211.6 ± 97.7U/L in controls. It was inferred that the serum LDH in acute leukemia patients were higher than that in OSCC patients, whereas the levels of salivary LDH in acute leukemia was lower than that of OSCC patients. The high level of serum LDH in AL was due to the type of metabolism of malignant leukemic cells causes the

difference in cell proliferation and turnover. In the oral cavity, the LDH activity is a net result of the enzymatic activity in gingival crevicular fluid being diluted in the saliva secretions with the added activity of the epithelial cells, leukocytes and bacteria to reach a value which is comparable to serum level.[40]

A biochemical study was conducted by **N. Subramanian, H. Mohana Krishnan, P. Venkatachalam and P.A.C. Kamatchi** to evaluate the usefulness of LDH isoenzyme on the diagnosis and treatment monitoring of cervical carcinoma. In this study 50 subjects diagnosed with cervical carcinoma were recruited for the study. Blood samples and tissue samples were obtained from all the subjects. The LDH isoenzyme pattern was analysed qualitatively using gel electrophoresis. Quantitative analysis of the enzyme LDH was carried out using UV spectrophotometer. The qualitative analysis of serum LDH revealed highest fraction of LDH1 isoenzyme (38.7%) and lowest activity of LDH5 isoenzyme (14.5%). The cervical tissue analysis showed that the LDH5 isoenzyme fraction was 80.1% and it was the highest while the LDH4 has the lowest activity of 27.8%. They found an increase in the serum level of LDH and an increase in the tissue activity of LDH in moderately differentiated cervical carcinoma and poorly differentiated cervical carcinoma patients. Therefore it was concluded that LDH is a potential biomarker for the diagnosis and treatment. [41]

Faruk Tas, Adnan Aydiner, Cumhur Demir and Erkan Topuz studied about the levels of serum LDH on the small cell lung cancer. It was reported that there was a significant association between the level of serum LDH and limited stage small cell lung cancer. The results showed that patients with serum LDH levels less than 470 IU/dl had worse prognosis. They concluded that high levels of serum LDH were indicators of poor prognosis of small cell lung cancer. [42]

Anup N. Nillawar, J. S. Bardapurkar and S. J. Bardapurkar studied the level of LDH as a biomarker for chronic obstructive pulmonary disease. It was shown that there was an average increase in the LDH activity in chronic obstructive pulmonary disease. This study also revealed that there was a decrease in serum LDH activity in current smokers. It was concluded that there is cellular alteration in the lung tissue after long time exposure to tobacco smoke which causes disease initiation.[43]

A biochemical study was conducted by **Pannuru Padmavathy, Vaddi Damodara Reddy and Nallanchakravarthula Varadacharyulu**. In this study serum biochemical profile was estimated in a group of 30 human male subjects. The subjects were under local diet and had the chronic habit of smoking 8-12 cigarettes per day for the past 7-10 years. The sample size was 60 with 30 volunteers in the cigarette smokers group and 30 participants in the non-smokers control group. The blood samples were collected and serum mineral content, enzymes and lipid profile was analysed for all the subjects. The results showed an alteration in the levels of serum electrolytes, with an increase in level of iron, phosphate, potassium and calcium ions. There was enhanced activity of serum enzymes like lactate dehydrogenase, alanine amino transferase, aspartate amino transferase and alkaline phosphatase. The serum lipoproteins, triglycerides and cholesterol showed a significant increase. The serum LDH was estimated to be 429 ± 22.47 for smokers when compared to non-smokers which was 320.16 ± 16.18 with a statistically significant difference. This increase in serum LDH was attributed to the fact that cigarette smoke induces skeletal muscle damage which can cause an increase circulatory LDH enzyme levels.[44]

In an in-vitro study conducted by **Katia Avezov, Abraham Z. Reznick and Dror Aizenbud**, the LDH enzyme activity in human saliva when exposed to cigarette

smoke was evaluated. The saliva for estimation was collected from seven male and female healthy non-smokers. The second source of the purified LDH 5 isoenzyme sample was obtained from rabbit muscle. In this study purified whole salivary LDH was exposed to different levels of cigarette smoke. The response of salivary enzyme was evaluated using spectrophotometry enzyme activity assay and gelatin electrophoretic densitometry. The salivary LDH enzyme activity showed a time and dose dependent decrease in its activity post cigarette smoke exposure. Within one hour of exposure to cigarette smoke the salivary LDH activity was decreased to 19.5% and in 3 hours time a reduction of 34% was seen in salivary LDH activity. The activity of all five isoenzymes are altered. [45]

Musutneei V studied about the salivary changes in diabetes patients. In the study the salivary levels of calcium, magnesium, phosphate, creatinine, aspartate aminotransferase, alanine aminotransferases and LDH were determined. The salivary samples were collected from insulin dependent diabetes mellitus patients and non-insulin dependent diabetes mellitus patients. The results showed that subjects with insulin dependent diabetes mellitus showed greater salivary concentrations of calcium and magnesium than the normal subjects and non-insulin dependent diabetes mellitus cases. Salivary concentrations of phosphate, aspartate aminotransferase and LDH were similar in the IDDM and NIDDM patients, but higher than those observed in normals. This study proved that inflammatory and degenerative changes in the salivary glands can be observed in cases of diabetes mellitus.[46]

Anders Linde and Anna Ljunggren studied the presence of LDH in pulpal tissue of deciduous teeth and impacted teeth. The LDH pattern were studied by using cellulose acetate paper electrophoresis. It was found that there was an increase in the LDH-3 and LDH-4 isoenzymes in the pulpal tissue. Since the LDH isoenzyme pattern of

a tissue indicates the relation between the capacities for aerobic and anaerobic metabolism. The increase in LDH proved the presence of a prominent anaerobic metabolism in human tooth pulp.

MATERIALS AND METHODS

SOURCE OF DATA

The present study was a comparative study done to evaluate the salivary lactate dehydrogenase (LDH) pattern in OSCC, OL, tobacco users and healthy controls. The study was conducted at Vivekanandha Dental College for Women, Elayampalayam, Tiruchengode Tk., Namakkal Dt. Tamil Nadu.

ETHICAL CLEARANCE

A detailed protocol about the aim and procedures of the present research was approved by the Institution Ethical Committee, Vivekanandha Dental College for Women.. The study was carried out after obtaining ethical clearance.

SAMPLE SIZE

The sample size was estimated using A Priori comparison test. The software used to calculate sample size was GPower version 3.1.9.2. It was done to estimate the number of subjects required for the study. The required sample size with 90% power was estimated as 23 per group with a total of 92 subjects. The details of the research was explained to all the participants and written consent was obtained from all of them.

The present study included a total of 109 subjects. The subjects were categorised into four groups.

Group	No. of cases	Criteria for inclusion
Group A	29	Patients diagnosed clinically and histopathologically with oral squamous cell carcinoma and who were not under any form of treatment were included in the group.
Group B	26	Patients with clinically and histopathologically diagnosed oral leukoplakia were included in the group.
Group C	24	Patients with the habit of smoking or chewing of tobacco for the past 2 years or more without any oral lesions were included in the group.
Group D	30	Patients without any chronic systemic illness or oral lesions and who did not have the habit of using tobacco in any form were included in the group.

INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria:

1. Clinically diagnosed and histopathologically confirmed cases of Oral squamous cell carcinoma.
2. Clinically diagnosed and histopathologically confirmed cases of oral leukoplakia
3. Patients with the habit of tobacco smoking or chewing for the past 2 years or more and without any identifiable oral lesions.
4. Healthy patients who were free from any oral or systemic diseases.

Exclusion criteria:

1. Patients suffering from systemic conditions like cardiovascular disease, anemia, liver, kidney and pancreatic diseases, blood dyscrasias, stroke, muscular dystrophy were excluded from the study.
2. Patient under drugs like anesthetics, narcotics and aspirin.
3. Individuals with mucosal lesions other than OL and OSCC were excluded.

Figure 4 - Clinical photograph showing malignancy in hard palate

Figure 5 – Post- operative photograph of incisional biopsy of malignancy in hard palate.

Figure 6 – clinical photograph of oral leukoplakia

Figure 7 – Post operative photograph of incisional biopsy of oral leukoplakia.

Figure 8 - Histopathology of Leukoplakia with no dysplasia

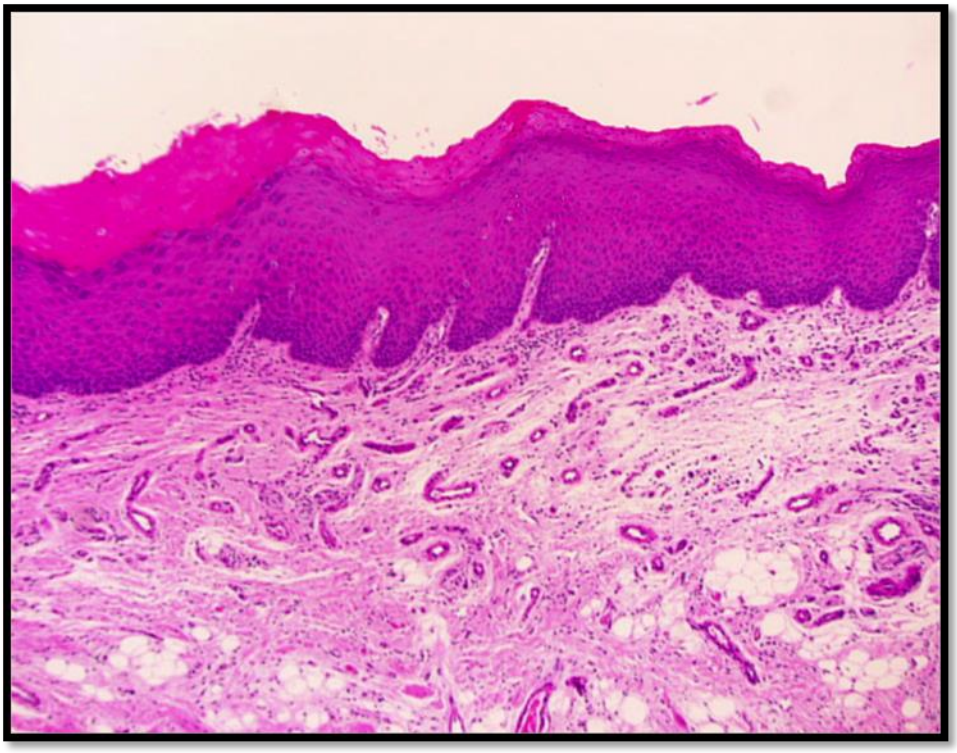


Figure 9- Histopathology of Leukoplakia with mild dysplasia

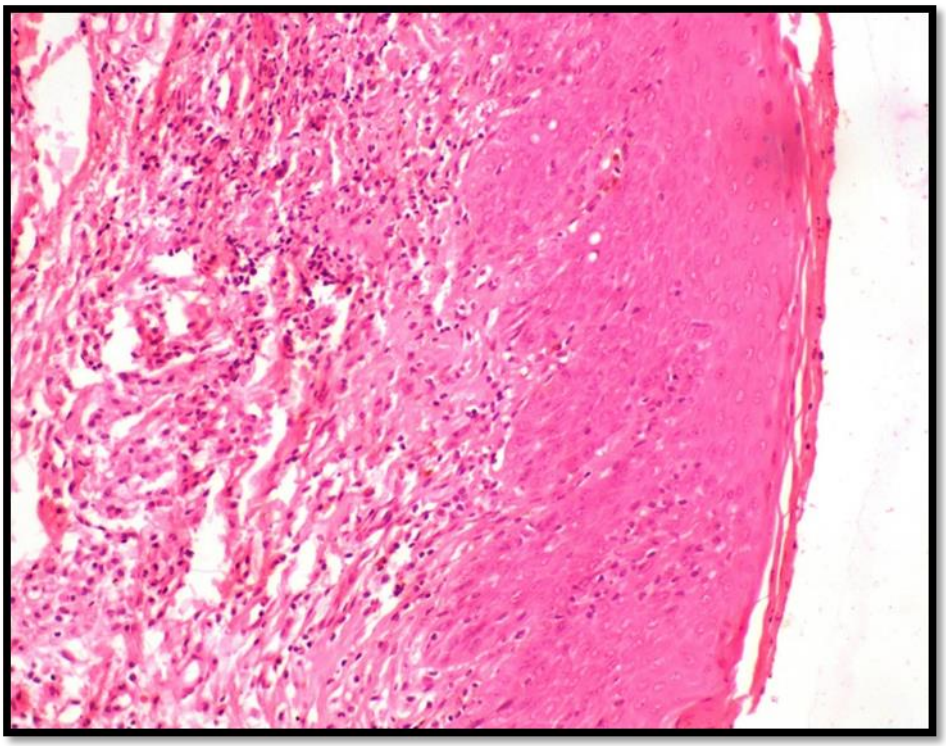


Figure 10- Histopathology of Leukoplakia with moderate dysplasia

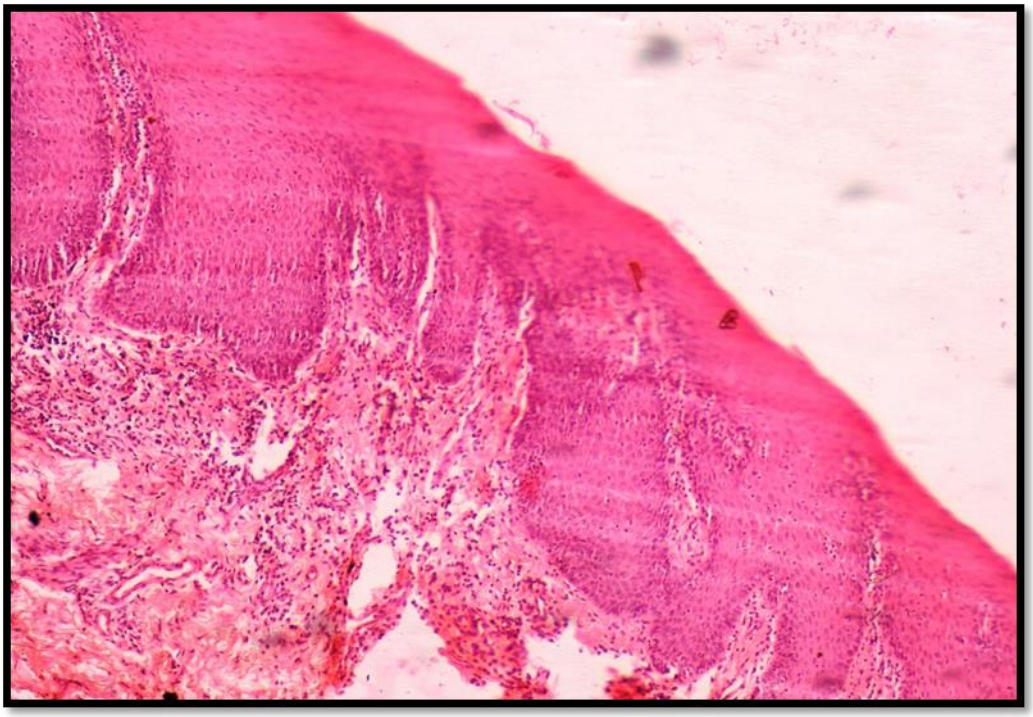


Figure 11- Histopathology of Leukoplakia with severe dysplasia

Figure 12- Histopathology of well differentiated squamous cell carcinoma

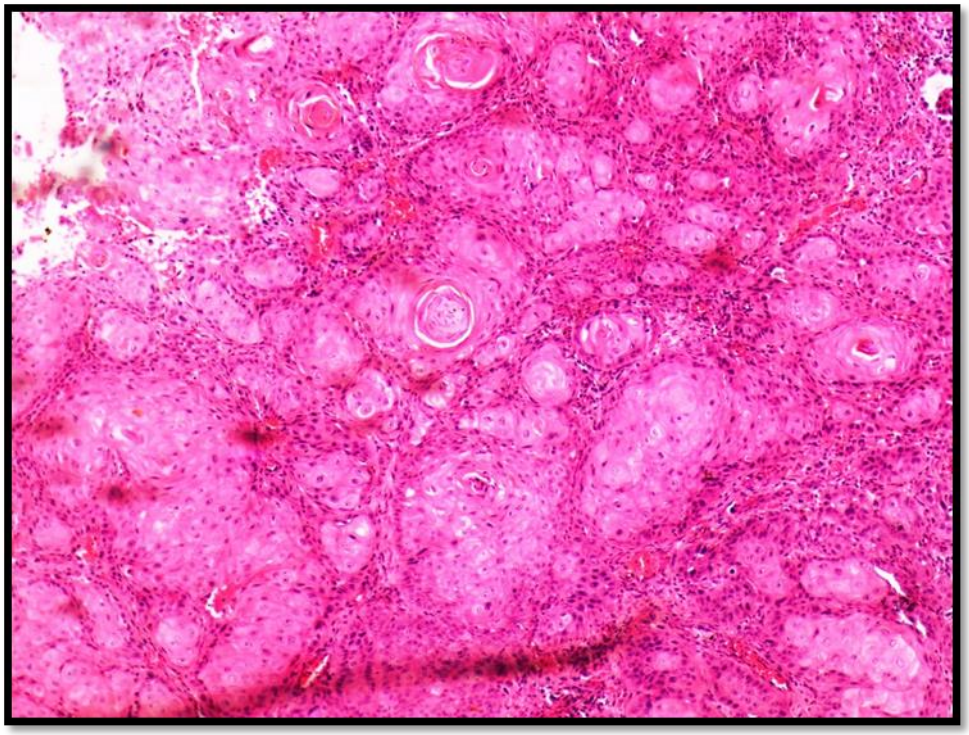


Figure 13- Histopathology of verrucous carcinoma

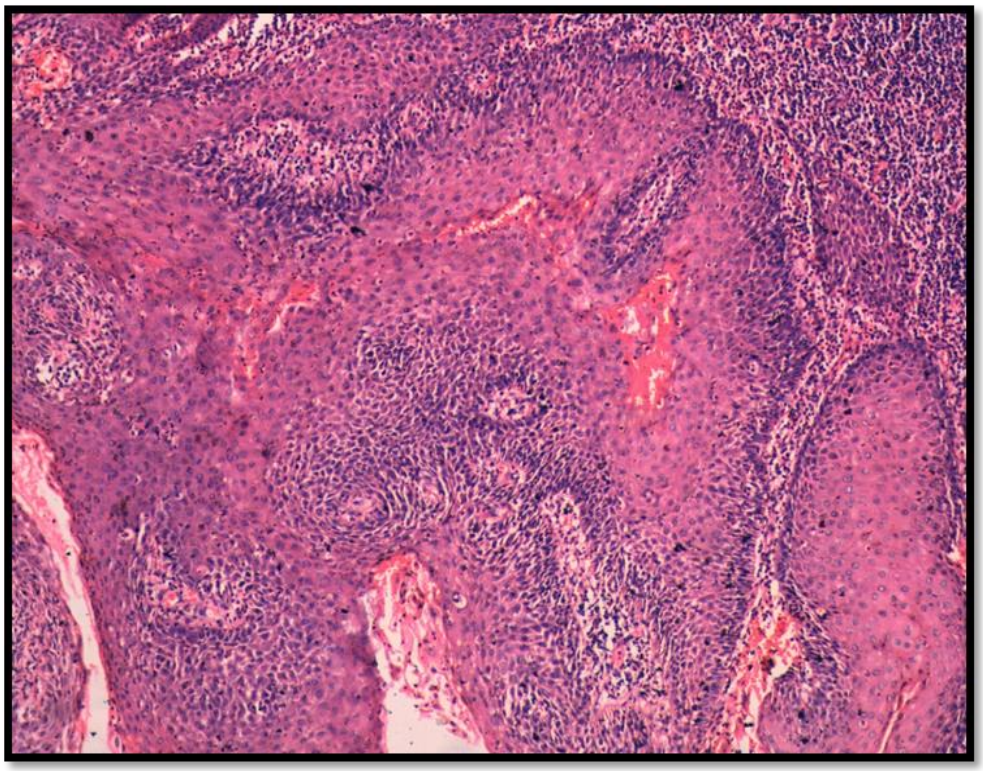


Figure 14 - Histopathology of Moderately differentiated squamous cell carcinoma

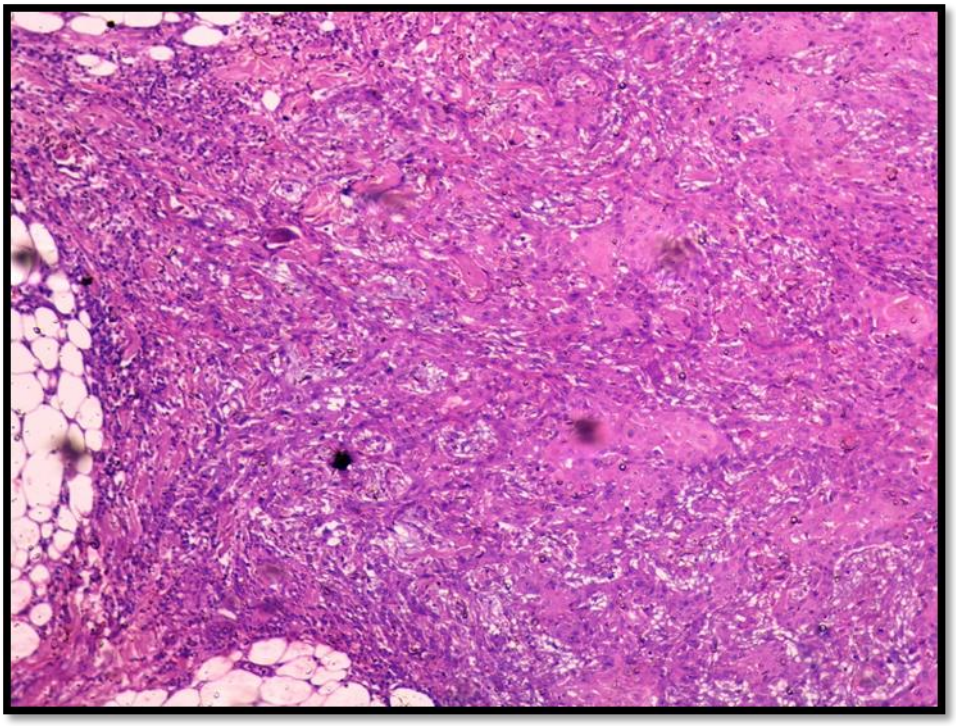
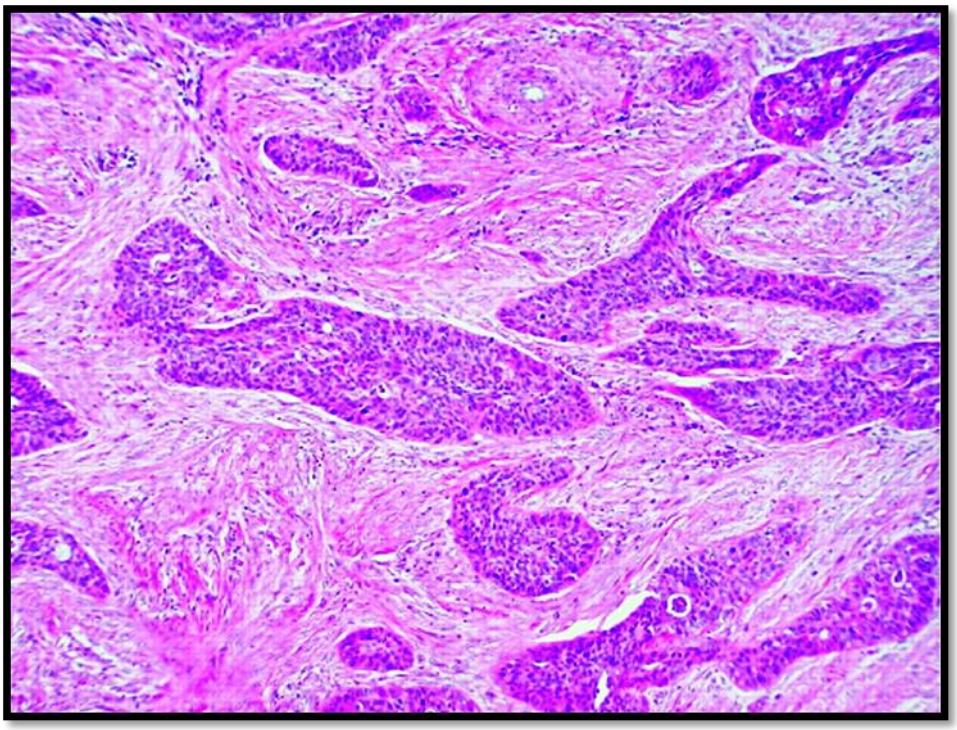


Figure 15 - Histopathology of poorly differentiated squamous cell carcinoma



Materials used:

Examination of subjects

1. Dental chair with good lighting attachment was used
2. Disposable gloves and mask
3. Stainless steel kidney tray
4. Disposable paper cups with water
5. Sterilized diagnostic instruments
    i. Mouth mirror
    ii. Straight probe
    iii. Explorer
    iv. Tweezers
    v. Cotton rolls
    vi. Gauze pads

Biopsy instruments

1. 3ml sterile disposable syringe with 26 gauge needle.
2. 2% lignocaine hydrochloride with 1: 100,000 adrenaline.
3. Biopsy tray
4. BP handle No. 4
5. Disposable No. 15 BP blade
6. 1 small surgical curved scissors
7. Artery forceps

8. Needle holder
9. Allis tissue forceps
10. A 3-0 Black braided silk suture
11. Curved suture needle (half circle) – 2 numbers
12. Sterile gauze and cotton
13. 10% neutral buffer formalin

Figure 16 - Sterilised Diagnostic Instruments

Figure 17- Biopsy Instruments

Collection of saliva samples:

1. Distilled water
2. Disposable paper cup
3. Sterile disposable 40 ml wide mouthed plastic containers with screw cap

Lab instruments:

1. Centrifugation machine
2. Eppendorf tubes
3. Test tubes
4. Test tube rack
5. Lactate dehydrogenase enzyme estimation kit
6. Semi - automatic analyser

Figure 18 - Disposable paper cup with distilled water and sterile plastic container

Figure 19 - Collection of saliva

Figure 20 - Centrifugation machine

Figure 21 - Micropipettes used for the analysis

Figure 22 – LDH enzyme estimation reagent

Figure 23 - Semi-automatic analyser

METHODOLOGY

Collection of data

The participants included in the study were thoroughly examined in the dental chair. The relevant information was obtained and written consent was obtained. Saliva was collected on the day of examination for the patients who belonged to the tobacco user group and healthy control group.

The patients who were clinically diagnosed with OSCC and OL underwent routine haematological investigations required for minor surgery. Biopsy was done under strict aseptic conditions and submitted for pathological diagnosis. On histopathological confirmation, saliva was collected.

Saliva collection

The patients were asked to refrain from eating, chewing gum or smoking for a period of 1 hour. They were comfortably seated in the dental chair. They were instructed to rinse the mouth with distilled water and unstimulated saliva was allowed to collect in the mouth for a period of 5 minutes and the patient was asked to spit it out.

Lab procedure

The collected saliva was centrifuged at 2500 rpm for 15 minutes. Then the resulting supernatant was subjected for further biochemical assay analysis using standard kit method. The samples was assayed using standard kit and the enzyme levels will be measured using semi-auto analyser.

STATISTICAL ANALYSIS

Data obtained was compiled in Microsoft Excel spreadsheet and a master chart was prepared. The data was subdivided according to the study groups. The statistical analysis was carried out using Statistical Package for Social Sciences software (SPSS version 16 IBM). The following statistical methods were applied in the study: -

ANOVA test

A one way ANOVA test was carried out to compare the data among the four groups.

The intra group comparison of the means between the histologically graded groups of oral squamous cell carcinoma and the grades of dysplasia was also done using one way ANOVA test.

Students' t – Test

The Student's T test was used to comparison between the mean value of LDH between males and females in each group was analysed using students' t test.

Pearson's correlation

This correlation test measure how variables or rank orders are related. Pearson's correlation coefficient is a measure of linear association. This was done to correlate the age with the LDH value in each group.

Chi square test

Chi square test was done to determine if there was any correlation between gender distribution and histological differentiating features of oral squamous cell carcinoma.

RESULTS

TABLE 1 - DISTRIBUTION OF SUBJECTS IN STUDY GROUPS

Group Name	No. of Subjects	Study group subjects
Group A	29	Oral squamous cell carcinoma.
Group B	26	Oral leukoplakia.
Group C	24	Tobacco user
Group D	30	Healthy controls
Total subjects		109

The table 1 shows the distribution of the subjects in each group.

Table 2- MEAN SALIVARY LDH LEVELS IN STUDY GROUPS

Study groups	Mean LDH in IU/L	SD	p Value
Group A – OSCC	1437.03	333.60	< 0.001**
Group B – OL	918.85	143.77	
Group C – Tobacco users	391.88	201.66	
Group D – Control	380.10	130.15	
	792.40	495.12	

** Highly Significant; SD- standard deviation

The table 2 shows the mean value of LDH in the study groups. The healthy control group has a mean value of 380.10 IU. The mean LDH value of the tobacco users group is 391.88IU. The mean LDH of the subjects diagnosed with oral leukoplakia was 918.85 IU, whereas the mean LDH of the subjects with oral squamous cell carcinoma was1437.03 IU. ANOVA test was done to compare the mean value. It showed highly significant difference as the P value was <0.001.

Table 3 MEAN SALIVARY LDH LEVELS IN MALES AND FEMALES FOR GROUP- A

GROUP A	Sex	N	Mean	SD	P value
LDH	Male	21	1410.10	366.04	0.491
	Female	8	1507.75	233.34	

The table 3 shows the mean LDH values in males and females in the group A patients (oral squamous cell carcinoma group). The mean value was 1410 IU for males and 1507.75 IU for females. The mean value in females is higher than males with an insignificant P value of 0.491.

Table -4 MEAN SALIVARY LDH LEVELS IN MALES AND FEMALES FOR GROUP -B

GROUP B	Sex	N	Mean	SD	P value
LDH	Male	23	938.00	138.20	0.058
	Female	3	772.00	108.25	

The table 4 shows the mean LDH values in males and females for Group B (leukoplakia group). The mean value was estimated as 938 IU for the male subjects and 772 IU for the female subjects. The mean value in male is higher than females with an insignificant P value of 0.058.

TABLE 5 –MEAN SALIVARY LDH LEVELS IN MALES AND FEMALES FOR GROUP - C

Group C	Sex	N	Mean	Std. Deviation	P value
LDH	Male	20	400.10	218.02	0.665
	Female	4	350.75	89.64	

The table 5 shows the mean LDH values in males and females for Group 3(tobacco users). It was estimated to be 400.10 IU for males and 350.75 IU for females. The mean value in male is higher than females with an insignificant P value of 0.665.

Table 6 -MEAN SALIVARY LDH LEVELS IN MALES AND FEMALES FOR GROUP D

Control	Sex	N	Mean	Std. Deviation	P
LDH	Male	15	427.27	129.25	0.045*
	Female	15	332.93	116.69	

The table 6 shows the mean LDH values in males and females in the control group. It was evaluated as 427.27 IU for females and 332.93 IU for males. The mean value in male is higher than females, with a significant P value of 0.045.

Table 7 – PEARSON'S CORRELATION OF SALIVARY LDH LEVELS WITH AGE FOR GROUP A

LDH		
Age	**R**	**P**
In Group A	-0.003	0.987

r- Pearson's correlation coefficient

The table 7 shows the Pearson's correlation for the LDH level when correlating with age in Group A - the carcinoma group. It shows a negative correlation of 0.003 with an insignificant P value of 0.987.

Table 8 PEARSON'S CORRELATION OF SALIVARY LDH LEVELS WITH AGE FOR GROUP B

LDH		
Age	**R**	**P**
In Group B	0.118	0.567

r- Pearson's correlation coefficient

The table 8 shows the Pearson's correlation for the LDH level when correlating with age in the leukoplakia group. It shows a positive correlation of 0.118 with an insignificant P value of 0.567.

TABLE -9 PEARSONS CORRELATION OF SALIVARY LDH WITH AGE FOR GROUP C

LDH		
Age	**R**	**P**
Group C	0.065	0.762

r- Pearson's correlation coefficient

The table 9 shows the Pearsons correlation for the LDH level when correlating with age in the tobacco groups. It shows a positive correlation value of 0.065 and has an insignificant P value of 0.762.

Table 10- PEARSONS CORRELATION OF SALIVARY LDH LEVELS WITH AGE FOR GROUP D

LDH		
Age	**R**	**P**
In Group D	-0.15	0.441

r- Pearson's correlation coefficient

The table 10 shows the Pearsons correlation for the LDH level when correlating with age in the control groups. It shows a negative correlation with age in the control group with an insignificant P value of 0.441.

Table 11 - INTRA GROUP COMPARISON OF MEAN SALIVARY LDH LEVELS AMONG THE TYPES OF DIFFERENTIATION IN GROUP A

Group A	N	Mean	SD	P
Poorly differentiated	4	1889.25	240.94	< 0.001**
Moderately differentiated	8	1615.88	169.65	
Well differentiated	15	1226.87	246.96	
Verrucous carcinoma	2	1393.50	382.54	
Total	29	1437.03	333.60	

N- Number of subjects

SD – standard deviation

P – P value

** statistically significant

The table 11 shows the intra group comparison of the mean value of LDH among the various histological differentiated stages of carcinoma. The 2 cases of verrucous carcinoma showed a mean value 1393.50 IU. In the study 15 cases of well differentiated carcinoma cases were present with a mean value of 1226.87 IU. The total number of moderately differentiated carcinoma cases were 8 with the mean value of 1615.88 IU. There were 4 cases of poorly differentiated squamous cell carcinoma with a mean value of 1889.25IU. This shows a progressive increase in the mean values of LDH from well differentiated to moderately differentiated to poorly differentiated.

Table -12 INTRA GROUP COMPARISON OF MEAN SALIVARY LDH LEVELS AMONG THE GRADING OF DYSPLASIA IN LEUKOPLAKIA GROUP

Leukoplakia	**N**	**Mean**	**SD**	**P**
No dysplasia	5	901.60	160.38	0.091
Mild dysplasia	13	866.77	96.73	
Moderate dysplasia	6	985.67	178.66	
Severe dysplasia	2	1100.00	113.14	
Total	26	918.85	143.77	

N- number of subjects

SD – standard deviation

P – P value

The table 12 shows the intra group comparison of the mean value of LDH among the histological grading of dysplasia in the leukoplakia group. There were 5 cases of leukoplakia with no dysplasia with a mean value of 901.60 IU. In the study 13 cases of leukoplakia with mild dysplasia were present with a mean value of 866.77IU. The total number of moderate dysplasia cases were 6 with the mean value of 985.67 IU. There were 2 cases of leukoplakia with severe dysplasia with a mean value of 1100IU. This shows a progressive increase in the mean values of LDH in the leukoplakia group from non-dysplastic cases to severe dysplastic cases.

TABLE 13 - CORRELATION OF SALIVARY LDH LEVEL IN DIFFERENT HISTOLOGICAL GRADES OF OSCC AND GENDER IN GROUP A

Histological grading of OSCC	Sex				Total		p
	Male		Female		N	%	
	N	%	N	%			
Poorly differentiated	2	10	2	25	4	14	0.050*
Moderately differentiated	6	29	2	25	8	28	
Well differentiated	13	62	2	25	15	52	
Verrucous carcinoma			2	25	2	7	
Total	21	100	8	100	29	100	

Table 13 shows the correlation with the histopathological grading of OSCC and gender in the group. Chi square test was done to determine the correlation. Out of 29 cases of OSCC only 8 subjects were females and 21 subjects were males. There was statistically significant relationship between the various grades of OSCC and gender distribution in our study with a P value of 0.050.

TABLE 14 - CORRELATION OF SALIVARY LDH LEVEL IN DIFFERENT DYSPLASTIC GRADES OF LEUKOPLAKIA AND GENDER IN GROUP B

Dysplasia	Sex				Total		p
	Male		Female		N	%	
	N	%	N	%			
No dysplasia	5	22			5	19	0.335
Mild dysplasia	10	43	3	100	13	50	
Moderate dysplasia	6	26			6	23	
Severe dysplasia	2	9			2	8	
Total	23	100	3	100	26	100	

Table 14 shows the Chi square test that was done to determine if there was any correlation between gender distribution and dysplastic features of leukoplakia. Out of 26 cases of leukoplakia only 3 subjects were females and 23 subjects were males. There was no statistically significant relationship between the various grades of dysplasia and gender distribution in our study.

TABLE 15 - SENSITIVITY AND SPECIFICITY OF THE TEST FOR CARCINOMA GROUP

Sensitivity (%)	96.55
Specificity (%)	100
Accuracy (%)	98.3
Positive predictive value (%)	100
Negative predictive value (%)	96.77

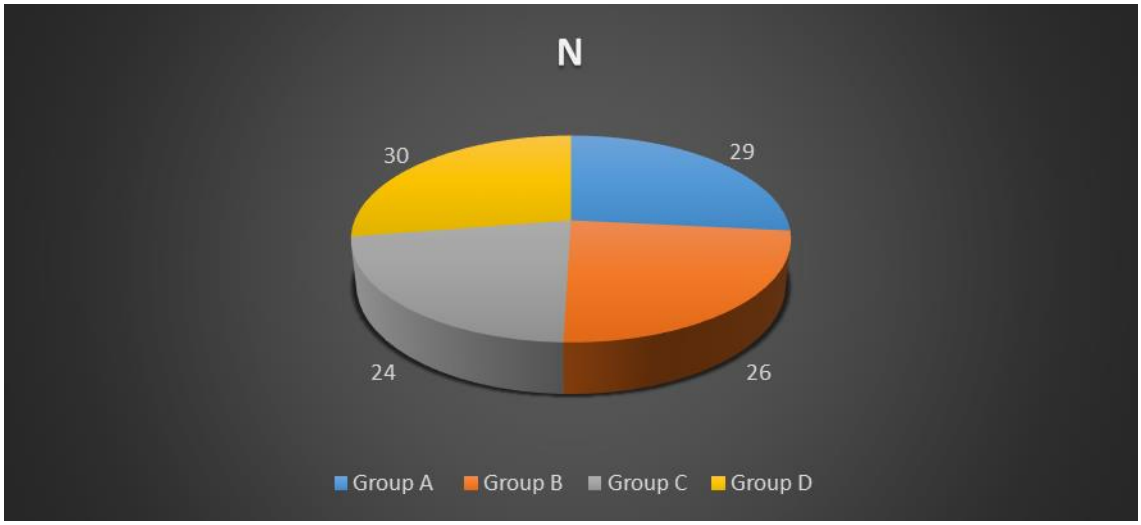
Table 15 shows the sensitivity, specificity and accuracy of salivary LDH to determine the diagnostic accuracy for carcinoma cases. It was found that the test is 96.55% sensitive and 100% specific. The accuracy of salivary LDH to detect OSCC is 98.3%. The positive predictive value was determined to be 1000% and the negative predictive value was 96.77% when compared with the control group.

TABLE 16 - SENSITIVITY AND SPECIFICITY OF THE TEST FOR LEUKOPLAKIA GROUP

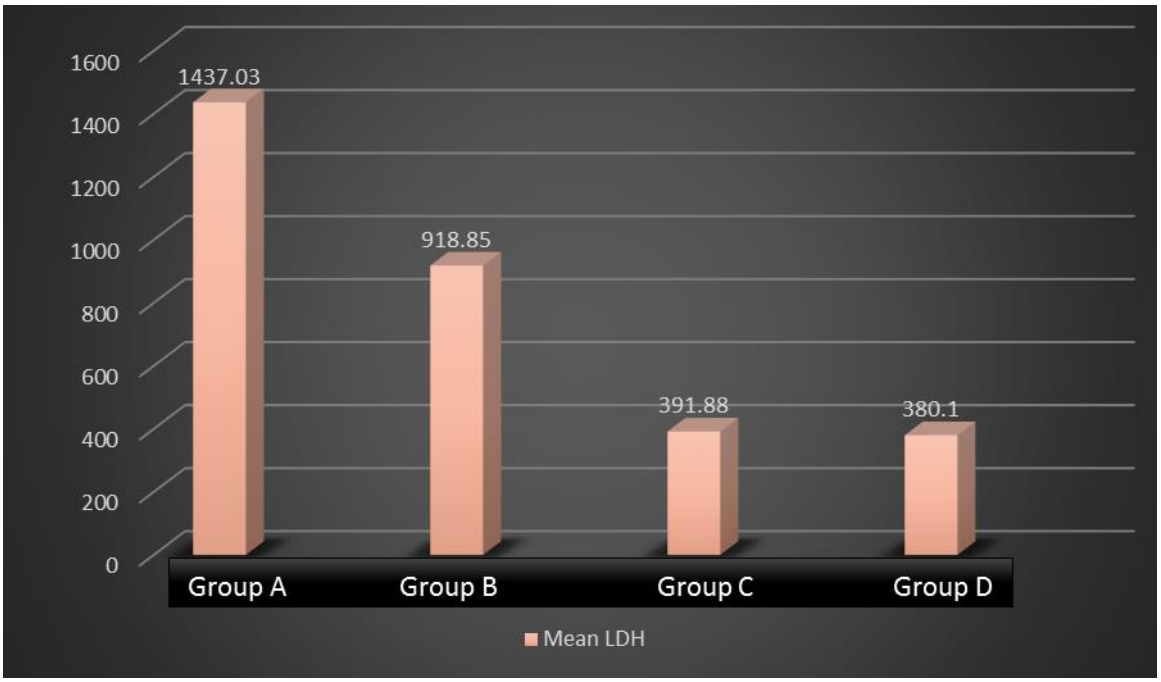
Sensitivity (%)	100
Specificity (%)	100
Accuracy (%)	100
Positive predictive value (%)	100
Negative predictive value (%)	100

Table 16 shows the sensitivity, specificity and accuracy of salivary LDH to determine the diagnostic accuracy for oral leukoplakia cases. It was found that the test is 100% sensitive and 100% specific. The accuracy of salivary LDH to detect OL is 100%. The positive predictive value was determined to be 1000% and the negative predictive value was 100% when compared with the control group.

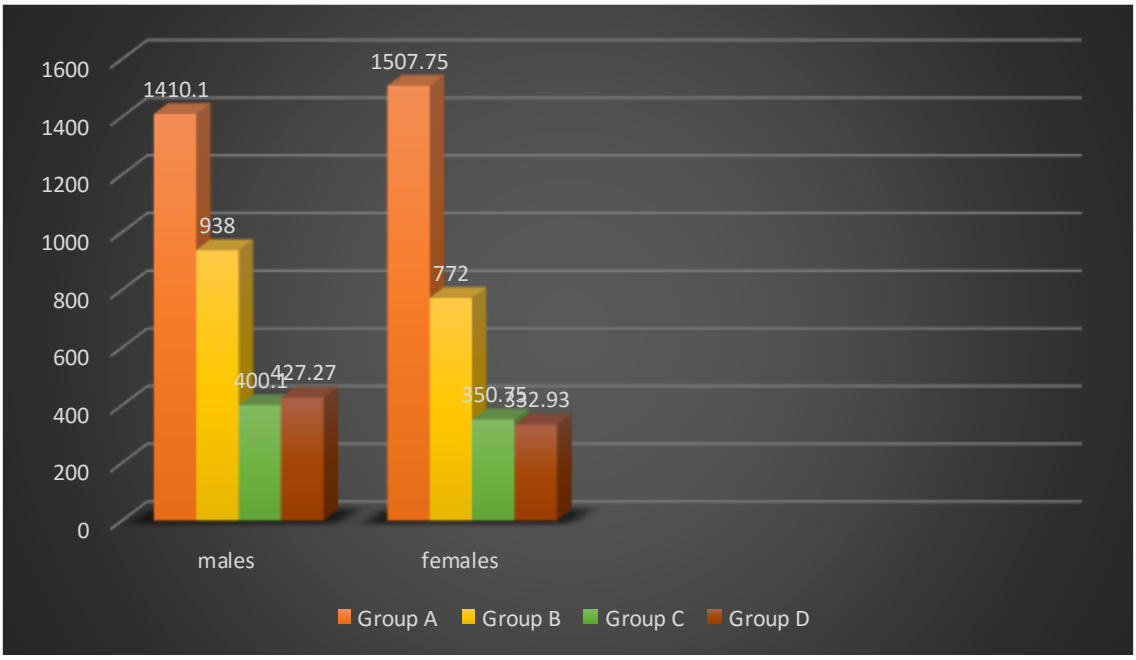
GRAPH 1- DISTRIBUTION OF SUBJECTS IN STUDY GROUP



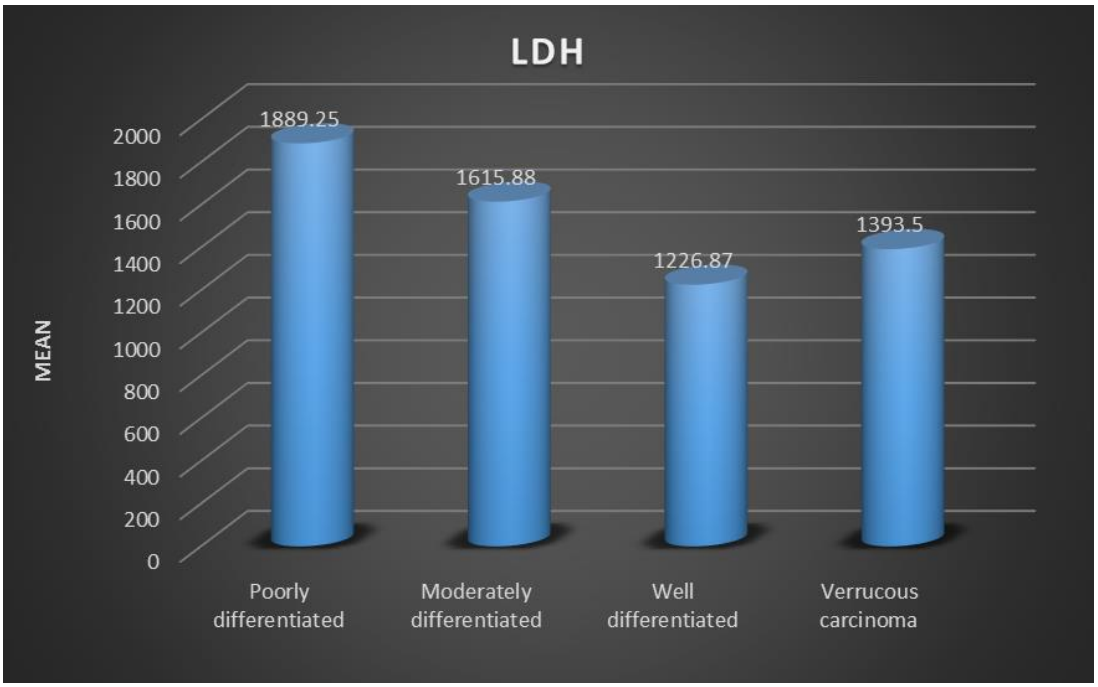
GRAPH 2- MEAN SALIVARY LDH IN STUDY GROUPS



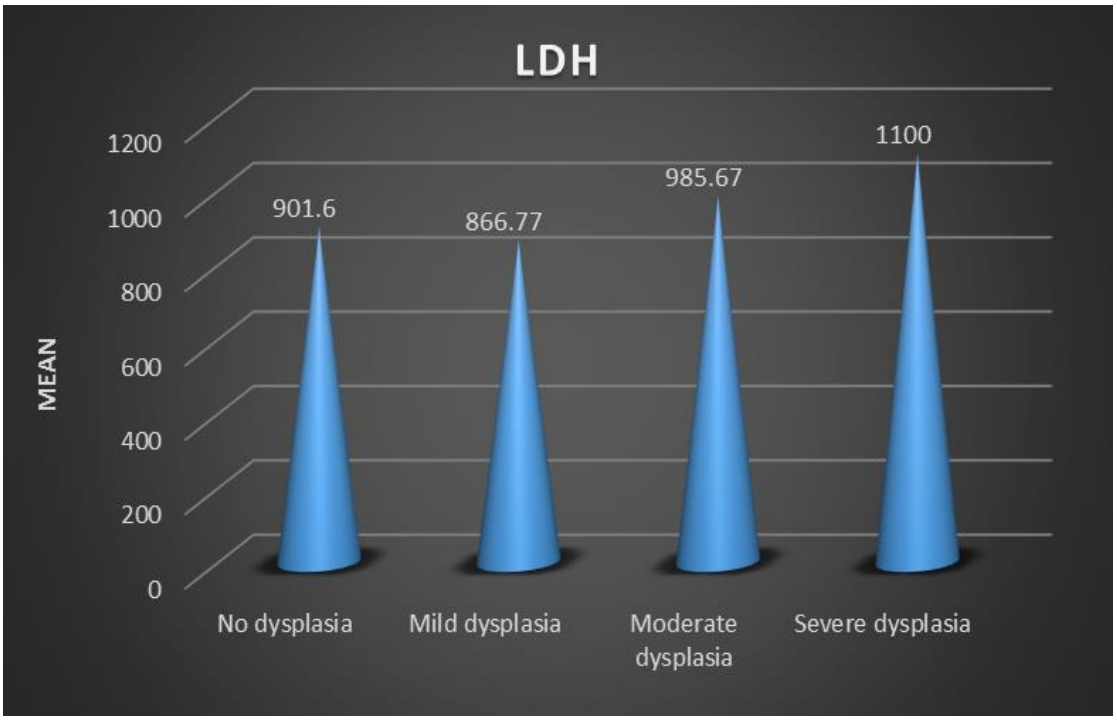
GRAPH 3- MEAN SALIVARY LDH OF MALES AND FEMALES IN STUDY GROUPS.



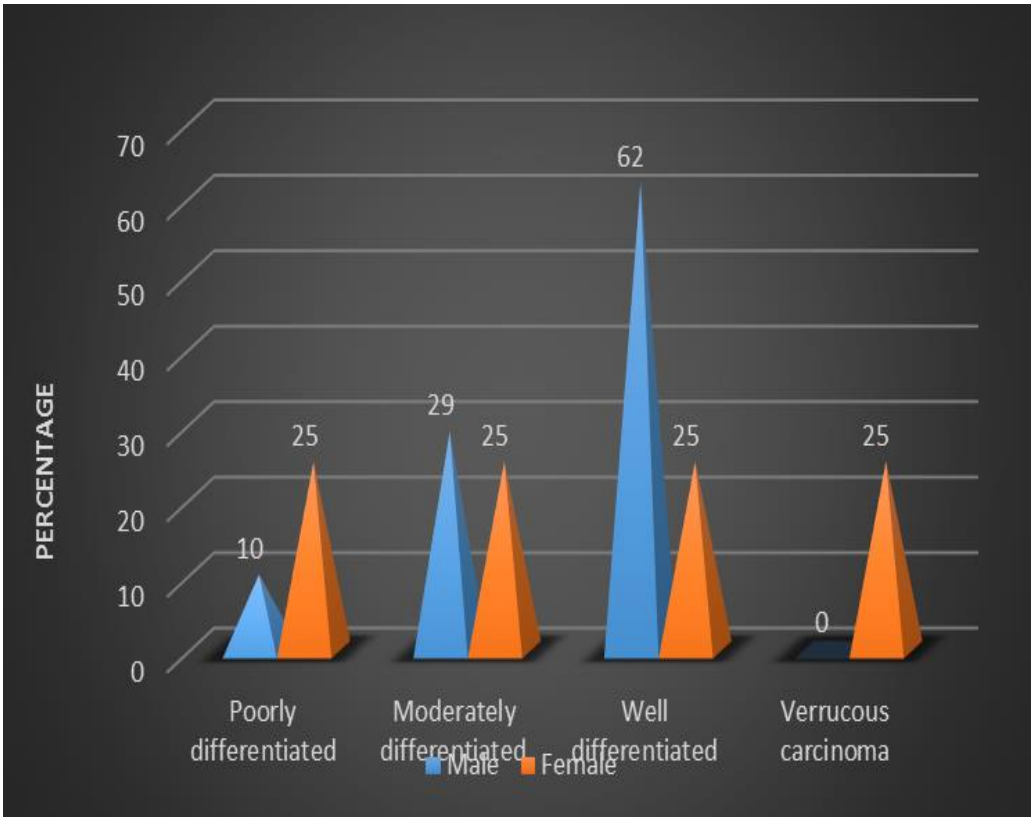
GRAPH 4 - INTRA GROUP SALIVARY LDH INGRADES OF OSCC



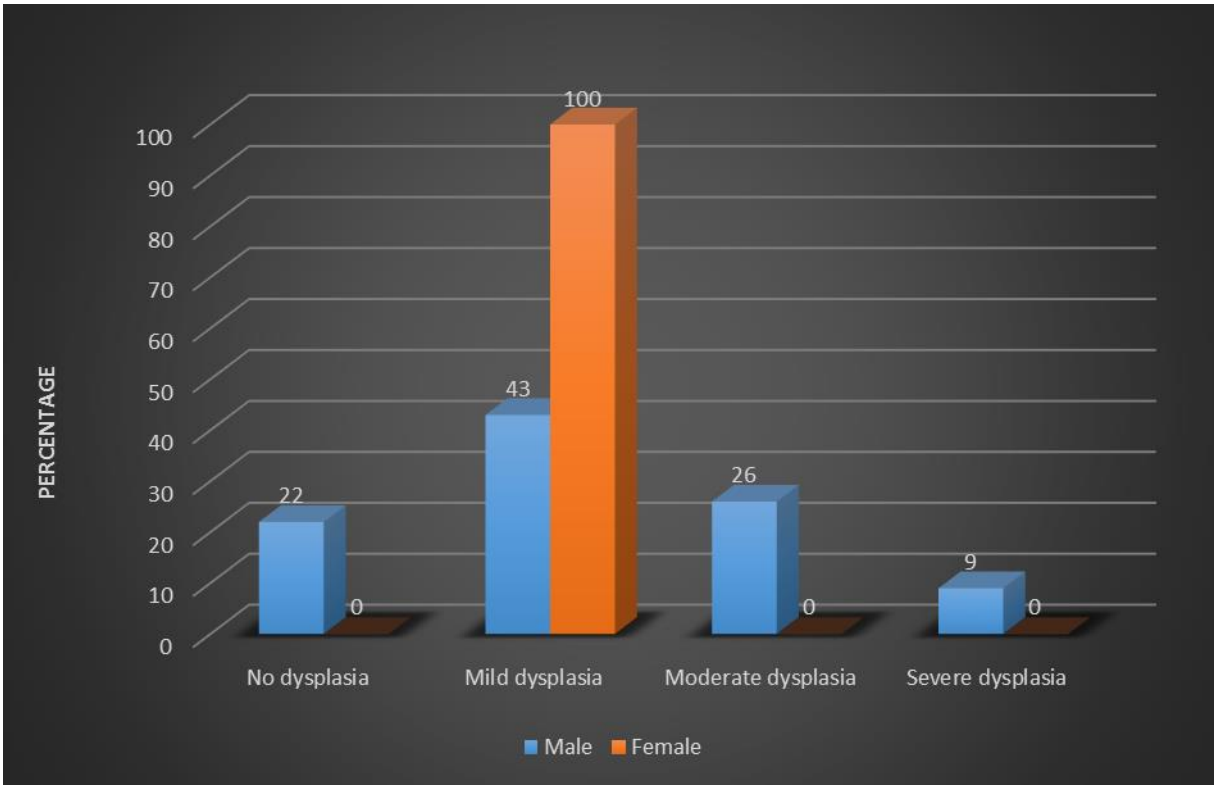
GRAPH 5- INTRA GROUP COMPARISON OF SALIVARY LDH IN LEUKOPLAKIA WITH VARIOUS GRADES OF DYSPLASIA



GRAPH 6 - CORRELATION BETWEEN DIFFERENT HISTOLOGICAL GRADES OF OSCC AND GENDER IN GROUP A



GRAPH 7- CORRELATION BETWEEN DIFFERENT GRADES OF LEUKOPLAKIA AND GENDER IN GROUP B



Discussion

The prevalence of cancer is widespread in India, 462408 male cancer patients and 517378 female cancer patients were the cases of cancer recorded in the year2010, with a total number of 979786 patients.[7] Currently, in the world, oral cancer is the fourth most common type of malignancy following lung, stomach and liver in males. It is considered to be the fifth common malignancy observed in females after cervix, breast, stomach and lung cancer. [7]

Carcinogenesis is the process of formation of cancer, where normal cells are transformed into malignant or cancer cells. This process is characterized by a continuous progressive changes at the cellular, genetic and epigenetic level that causes reprogramming of a cell to undergo uncontrolled cell multiplication thereby forming a malignant mass. The process of carcinogenesis in the oral cavity is a highly complex multifactorial process that occurs when epithelial cells are affected by many genetic changes.[48]

Oral leukoplakia is a commonly encountered premalignant lesion. OL can transform into a dysplastic lesion, even invasive, with no evidence of change in its clinical appearance. It is estimated that in a year about 54,642,560 to 87,046,868 cases of OL are prevalent. Out of which 377,034 to 1,767,051 cases transform to OC.[10]

A large majority of OSCCs arise de novo, without preceding visible changes in the mucosa. Additionally, OSCC may progress from early to advanced stages within a very short time perhaps a few weeks, which hamper early diagnosis for OSCC.

Nowadays, molecular biology techniques are being developed and used to diagnose oral precancerous and cancerous lesions. This may significantly improve the early detection of malignant alterations that are not visible under the microscope. This

can aid identification of subjects who are at a higher risk of developing oral cancer. [14]There are several risk factors associated with the occurrence of OC. Chemical agents like tobacco and alcohol, infectious agents like human papillomavirus (HPV), hyperplastic candidiasis and syphilis, dental causes and dietary practices have shown to significantly increase the incidence of OSCC. [49]

The practices of tobacco usage are prevalent in various forms such as smoking, chewing as plain tobacco leaves and combining it with the betel quid and chewing etc. Though there is extensive awareness created among the people about the harmful effects of smoking, the practices still prevail. This has shown to be a major risk factor leading to OC. Tobacco smoke has high levels of oxidants. [50]

It has been stated that, there is an increased production of reactive oxygen species due to smoking and it may exceed the capacity of the host oxidant defence system, causing severe oxidative damage.[51] The practice of tobacco usage appears to be closely associated with the development of cancer and precancerous lesion like oral leukoplakia. It was estimated that more than 80% of patients with OL were smokers.[18]

In the present research, the study population consisted of a total of 109 subjects who were divided into 4 groups. The total number of healthy controls included in the study were 30. The number of subjects in the tobacco users group without any oral lesions were 24. The subjects with the oral lesion of leukoplakia were 26. The number of patients with oral squamous cell carcinoma were 29.

In the present study we evaluated the salivary LDH enzyme levels in patients with OSCC, OL, tobacco users and healthy controls. We found out that the mean value of salivary LDH progressively increased from healthy controls to tobacco users followed by OL and OSCC. On comparison of the mean values in the 4 groups there was a highly

significant difference with a P value of <0.001. The mean level of salivary LDH in our study for OL was 918.85±143.77IU/L and for OSCC was 1437.03± 333.60IU/L.

In a study conducted by Patel S et al who had evaluated the salivary LDH level in OL and OSCC, the mean value for control group was 261.16±75.851IU/L, OL group was 497.00±100.404 IU/L and for the OSCC group was 686.40±81.752 with statistically significant P value.[26]

In another study by Joshi PS et al the salivary LDH isoenzyme pattern in OSCC and OL was determined. The total salivary LDH was estimated to be 267.2 IU/L in the control group, 519.3667IU/L in the OL group and 788.7333IU/L in the OSCC group.[29]

In the study conducted by Dhivyalakshmi M et al. The mean salivary LDH was estimated to be 79.70 IU/L for control, 102.54 IU/L for OL and 268.57 IU/L for OSCC. [30]

In a study conducted by Shishir Ram Shetty *et al*, the mean salivary LDH was 79.50 ± 4.67 IU/L for healthy controls, 136.46 ± 3.36 IU/L for OL and 148.77 ± 4.83 IU/L for OSCC.[33]

There is a progressive increase in the mean value from OL to OSCC in all the above studies which is similar to the present study. But the mean values obtained in the current study are considerably higher in the present study population.

In the present study the total number of OSCC cases were 29. The intra group comparison of the mean value of LDH was done for the various histologically differentiated grades of OSCC. There were 2 cases of verrucous carcinoma which showed a mean value 1393.50 IU± 382.54. In the study 15 cases of well differentiated carcinoma cases were present with a mean value of 1226.87 IU± 246.96. The total

number of moderately differentiated carcinoma cases were 8 with the mean value of 1615.88 IU± 169.65. There were 4 cases of poorly differentiated squamous cell carcinoma with a mean value of 1889.25IU± 240.94. This shows a progressive increase in the mean values of LDH from well differentiated to verrucous carcinoma to moderately differentiated to poorly differentiated.

In a similar study by Patel S et al who compared the LDH value in the histological grades of OSCC they found that the intragroup levels of salivary LDH in the OSCC group was estimated as 745.53±98.403 IU/L for well differentiated OSCC, 799.129±89.404 for moderately differentiated OSCC and 828.25±79.752 for poorly differentiated OSCC with a statistically insignificant P value. The mean salivary LDH are higher in our study population.[26]

In another study conducted by Pereira T et al, they had estimated the serum lactate dehydrogenase level in patients with oral premalignant disorders and oral squamous cell carcinoma. The serum LDH was estimated to be 339.90 IU/L for the control group, 488.67 IU/L for leukoplakia, 743.30 IU/L for well differentiated OSCC and 988.50 IU/L for moderately differentiated OSCC. There was a progressive rise in the serum LDH level from control group to OSCC group. [25]

In yet another research done by Audrey M D'Cruz et al, they had conducted a case-control study to compare the levels of salivary LDH in various pathological differentiation of OSCC patients. The mean salivary LDH levels was 117.33 ± 19.37 IU/L in the control group , 355.83 ± 16.73 IU/L in the well-differentiated OSCC group, 484.18 ± 25.84 IU/L in the moderately differentiated OSCC group, and 620.35 ± 18.69 IU/L in the poorly differentiated OSCC group. There was a statistically significant difference between the three groups. It was analysed that the source of salivary LDH was

largely non glandular and was due to the direct diffusion of the enzyme from the epithelial cells. Therefore, salivary LDH can be considered as an important biomarker for detection of OSCC. [31]

In all the above studies there is a progressive increase in the mean value of LDH from well differentiated to moderately differentiated and poorly differentiated OSCC groups. In the present study the results are similar to the other studies but the mean value of salivary LDH is greater. There were 2 cases of verrucous carcinoma which were also included in our study. This was not included in any other studies. The mean value of verrucous carcinoma was higher that well differentiated OSCC.

In the study done by Kavya Shree Lokesh, Jayanthi Kannabiran, Mahesh Dathu they found that the mean value of salivary LDH for healthy controls was 497.00IU/L whereas the OSCC cases showed a mean value of 1225.40IU/L. Based on the pathological differentiation, the salivary LDH levels for the various grades of OSCC were compared and the mean values was 1049.07 IU/L for well differentiated, 1309.50IU/L for moderately differentiated and 1586.20IU/L for poorly differentiated carcinoma. The results of this study is very similar to our present study. [34]

In the present study, total number of cases in the leukoplakia group was 26. Out of which 5 cases showed no dysplasia, 13 cases showed mild dysplasia, 6 cases showed moderate dysplasia and 2 cases showed severe dysplasia. The intra group comparison of the mean salivary lactate dehydrogenase levels for the different grading of leukoplakia was done. Salivary LDH in the group without dysplasia was 901.30$\pm$160.38IU/L, in the group with mild dysplasia was 866.77$\pm$96.73IU/L, in the group with moderate dysplasia was 985.67$\pm$178.66IU/L and in the group with severe dysplasia was 1100.00$\pm$113.14. The mean value of salivary LDH shows a progressive increase from the mild dysplasia

group to moderate dysplasia group to the severe dysplasia group with a statistically insignificant P value of 0.091. The mean value of salivary LDH in the group without dysplasia is slightly higher than that of the mild dysplasia group. This can be attributed to the varying flow rate of saliva in different temperature conditions, hydration status, stress, age and hereditary influence.[52]

In a study done by Joshi PS et al., the LDH isoenzyme pattern was evaluated for the dysplastic grades of leukoplakia. In the leukoplakia cases with no dysplasia, the isoenzyme LDH 1 was 40.024±11.389IU/L, LDH 2 was 65.415±4.797 IU/L, LDH 3 was 79.356±6.541, LDH 4 was 141.963±10.362 and LDH 5 was estimated as 206.673±13.634. In leukoplakia cases with mild dysplasia, the isoenzyme levels of LDH 1 was 47.752±8.896IU/L, LDH 2 was 57.915±8.525IU/L, LDH 3 was 70.446±6.555IU/L, LDH 4 was 128.342±11.201IU/L and LDH 5 was 172.743±20.182IU/L. In leukoplakia cases with moderate dysplasia, the isoenzyme pattern was 63.285±30.861IU/L for LDH 1, 84.474±24.258IU/L for LDH 2, 75.141±5.082IU/L for LDH 3, 172.242±12.459IU/L for LDH 4 and 225.858±5.742IU/L for LDH 5. In leukoplakia with severe dysplasia cases, LDH 1 was estimated as 46.35IU/L, LDH 2 was 69.75IU/L, LDH 3 was 58.95 IU/L, LDH 4 was 133.65IU/L and 141.3IU/L. There was progressive increase in the isoenzyme LDH 5, LDH 4, LDH 3 and LDH 2 in leukoplakia cases.[29]

In the present study, the salivary LDH was estimated for the subjects using tobacco in chewable and smoking form and who did not show any clinically detectable oral lesions. The mean LDH value was estimated as 391.88 $\pm$ 201.66IU/L when compared to healthy controls with mean LDH of 380.10$\pm$130.15IU/L. This is to our knowledge a first study undertaken to evaluate the salivary LDH in tobacco users and to compare the value with oral premalignant and malignant disorders.

In a study by Padmavathy et al, they had estimated the serum levels of LDH in chronic cigarette smokers i.e., smoking 8-12 cigarettes per day for a period of 7-10 years. The results showed that LDH level in serum was 429±22.47 for smokers when compared to non-smokers which was 320.16±16.18 with a statistically significant difference. In this study the increase in serum LDH was due to the fact that cigarette smoke causes increased skeletal muscle damage which can elevate the circulatory LDH enzyme levels.[44]

In another in-vitro study conducted by Katia Avezov et al, the LDH enzyme activity in human saliva when exposed to cigarette smoke was evaluated. In this study purified whole saliva was subjected to different levels of cigarette smoke. The salivary LDH enzyme activity showed a time and dose dependent decrease in its activity post cigarette smoke exposure. Within one hour of exposure to cigarette smoke the salivary LDH activity was decreased to 19.5% and in 3 hours' time a reduction of 34% was seen in salivary LDH activity.[45]

In this study, the sensitivity and specificity of salivary to detect OSCC is 98.3%. The positive predictive value was determined to be 100.0% and the negative predictive value was 96.77% when compared with the control group. It was also found to be 100% sensitive and 100% specific to detect OL. The positive predictive value was determined to be 100% and the negative predictive value was 100% when compared with the control group.

In our present study we have evaluated the salivary biomarker- lactate dehydrogenase enzyme in oral squamous cell carcinoma, premalignant lesion- leukoplakia and in tobacco users. We found out that the salivary LDH levels can serve as a reliable biomarker to predict malignant conditions. The drawback in our study is that

equal distribution of subjects in each histological variant of OSCC and in each grading of dysplasia in the leukoplakia group was not done. Also, for the tobacco users the pack-year determination of tobacco smoking was not followed as the subject who smoked or chewed tobacco was selected for the study. Similar study with greater sample size can be carried out for better validation of the results. The evaluation of salivary biomarker LDH can be a non-invasive reliable tool for the early detection of oral premalignancy and malignancy.

SUMMARY

A study titled "Evaluation of Salivary Biomarker – lactate dehydrogenase in tobacco users, oral leukoplakia and oral squamous cell carcinoma-a comparative study" was conducted in the Department of Oral Medicine & Radiology at Vivekanandha Dental College for Women between 2015 and 2017. It is a comparative study with 29 cases in the OSCC group, 26 cases in the OL group, 24 cases in the tobacco users group and 30 healthy patients in the control group. The OL and OSCC cases were clinically diagnosed and histopathologically confirmed before inclusion in the study.

The whole unstimulated saliva was collected and centrifuged immediately. The supernatant was subjected to biochemical analysis using semi-automatic analyser. The salivary LDH was estimated for all the samples.

The following inferences were made in the present study:

- The salivary LDH level was the highest in the patients with OSCC, followed by OL, tobacco users and healthy controls. The difference between the groups were highly significant. (p Value<0.001)
- A higher mean salivary LDH value was seen in males when compared to females in OSCC group which was statistically insignificant. (p Value 0.491)
- A higher mean salivary LDH value was seen in males when compared to females in OL group which was statistically insignificant.(p Value 0.058)
- A non-significantly higher mean salivary LDH value was seen in males when compared to females in tobacco users group.(p Value 0.665)

- A significantly higher mean salivary LDH value was seen in males when compared to females in healthy control group.(p Value 0.045)
- No significant correlation was found between the age and salivary LDH level in this study.
- A statistically significant difference was found between the histopathological grades of OSCC. (p Value <0.001)
- An insignificant correlation was found between the grades of dysplasia in Leukoplakia. (p Value 0.091)
- The sensitivity and specificity tests showed that the test was highly sensitive and specific to detect oral leukoplakia and oral squamous cell carcinoma.

In the future, researches should be done by considering larger sample size. Follow up of the patients was not done in the study.

Further research by including the follow up salivary LDH status can yield better understanding on the salivary biomarker level in premalignant and malignant states.

CONCLUSION

This study was carried out to assess the validity of salivary biomarker, lactate dehydrogenase for the detection of oral premalignancy and malignancy changes.

The results suggest that there is a significant difference in the mean salivary LDH values between the 4 study groups. The salivary LDH is highest in the OSCC group followed by OL group. The salivary LDH was slightly higher in smokers than in healthy controls. Therefore, salivary LDH can be used as a biomarker for the detection of oral cancer and oral leukoplakia.

The detection of this enzyme can serve a potent diagnostic aid for the early detection of malignant disease of oral cavity. Further studies are required to validate the results and use the test in the future.

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STUDY PROFORMA

"EVALUATION OF SALIVARY BIOMARKER – LACTATE DEHYDROGENASE IN TOBACCO USERS, ORAL LEUKOPLAKIA AND ORAL SQUAMOUS CELL CARCINOMA-A COMPARATIVE STUDY"

Name of the patient:

Age / Sex:

Address and contact No. :

Chief complaint:

Personal history:

Smoking Habit: Bidi/ Cigarette/ Chewable form of tobacco

Other habits, if any:

Duration of habit :

Frequency of habit:

Description of the lesion (if applicable):

Provisional diagnosis:

Histopathological diagnosis(if applicable):

INFORMATION SHEET AND CONSENT FORM

This informed consent is for the patients who attend the Vivekanandha Dental College for Women and who are willing to participate in research titled "Evaluation of salivary biomarkers: lactate dehydrogenase in tobacco users, oral leukoplakia, oral squamous cell carcinoma and healthy controls- A comparative study.

We are doing a research on tobacco usage, oral leukoplakia and oral cancer. I am going to give you information and invite you to be a part of this study. This involves collection of 5-10 ml unstimulated saliva and its further analysis. Tissue biopsy will be done to confirm the provisional diagnosis in case of pathology.

Informed consent form for patients

I consent voluntarily to participate as a participant in this research

Signature of participant / thumb impression **Date**

MASTER CHART FOR STUDY GROUP A – OSCC GROUP

S.no.	Patient Name	Age	Gender	Differentiation	LDH
1	Gopikrishnan	52	Male	Well Differentiated	980
2	Ramya	69	Female	Moderately Differentiated	1423
3	Arun Raj	58	Male	Well Differentiated	1100
4	Mariyammal	50	Female	Verrucous Carcinoma	1664
5	Muthumayammal	60	Female	Verrucous Carcinoma	1123
6	Pavalayee	59	Female	Well Differentiated	1280
7	Muthugoundan	55	Male	Well Differentiated	1008
8	Kandhasamy	60	Male	Moderately Differentiated	1669
9	Latha	60	Female	Well Differentiated	1423
10	Anukumar	50	Male	Well Differentiated	908
11	Chenniyappan	60	Male	Well Differentiated	1400
12	Kannaya	71	Male	Well Differentiated	1280
13	Saradha	45	Female	Moderately Differentiated	1655
14	Kandhasamy	67	Male	Well Differentiated	1350
15	Kesavan	40	Male	Moderately Differentiated	1750
16	Ravi	50	Male	Well Differentiated	1080
17	Ramesh	45	Male	Moderately Differentiated	1450
18	Muthukumar	60	Male	Moderately Differentiated	1855
19	Lakshmi	55	Female	Poorly Differentiated	1803
20	Sengondan	69	Male	Moderately Differentiated	1402
21	Rangasamy	49	Male	Well Differentiated	1720
22	Senthamizhan	49	Male	Well Differentiated	1654
23	Govindhasamy	62	Male	Poorly Differentiated	1823
24	Lavanya	50	Female	Poorly Differentiated	1691
25	Kumaresan	69	Male	Poorly Differentiated	2240
26	Krishnan	69	Male	Moderately Differentiated	1723
27	Velkumaran	62	Male	Well Differentiated	989
28	Palaniyappan	62	Male	Well Differentiated	1047
29	Ramakrishnan	60	Male	Well Differentiated	1184

MASTER CHART FOR STUDY GROUP B – OL GROUP

S. No	Patient Name	Age	Gender	Leukoplakia- Dysplasia	LDH Level
1	Venkatachalam	43	Male	Moderate Dysplasia	950
2	Marayee	50	Female	Mild Dysplasia	709
3	Ramasamy	40	Male	Moderate Dysplasia	670
4	Selvaraj	63	Male	Moderate Dysplasia	1007
5	Arumugam	49	Male	Moderate Dysplasia	980
6	Subramaniam	63	Male	Mild Dysplasia	871
7	Sukumar	30	Male	Mild Dysplasia	764
8	Soundarajan	50	Male	Severe Dysplasia	1020
9	Kuppusamy	61	Male	Mild Dysplasia	940
10	Subramaniyam	65	Male	Mild Dysplasia	780
11	Jayaraman	56	Male	Mild Dysplasia	876
12	Sivaraj	60	Male	No Dysplasia	880
13	Dharmalingam	65	Male	No Dysplasia	1160
14	Subramaniam	65	Male	Mild Dysplasia	970
15	Selvam	68	Male	No Dysplasia	898
16	Mani	29	Male	Moderate Dysplasia	1123
17	Raghav	34	Male	Mild Dysplasia	947
18	Pangarusamy	70	Male	Mild Dysplasia	1004
19	Velusamy	62	Male	No Dysplasia	850
20	Kulandhasamy	70	Male	Severe Dysplasia	1180
21	Kandhangi	63	Female	Mild Dysplasia	710
22	Ramachandran	53	Male	No Dysplasia	720
23	Nagappan	50	Male	Mild Dysplasia	890
24	Palaniyappan	66	Male	Mild Dysplasia	910
25	Rosy	58	Female	Mild Dysplasia	897
26	Ramu	50	Male	Moderate Dysplasia	1184

MASTER CHART FOR STUDY GROUP C – TOBACCO USERS

S.no	patient name	Age	Gender	LDH level
1	Kannan	30	Male	762
2	Karthick	37	Male	321
3	Murugappan	50	Male	417
4	Manoharan	34	Male	721
5	Rajendran	41	Male	314
6	Ramasamy	66	Male	675
7	Sekar	42	Male	223
8	Arumugam	58	Male	197
9	Vishwanathan	40	Male	309
10	Rajagounder	50	Male	162
11	Mohan	30	Male	150
12	Ramasamy	40	Male	179
13	Rangasamy	70	Male	190
14	Selvaraj	63	Male	150
15	Papathi	52	Female	340
16	Durga	45	Female	420
17	Nandhitha	47	Female	415
18	Kavitha	38	Female	228
19	Sembaram	60	Male	718
20	Kannayan	45	Male	465
21	Maniram	40	Male	324
22	Richard	30	Male	470
23	Subramaniyam	65	Male	675
24	Chinnappan	60	Male	580

MASTER VHART FOR STUDY GROUP D- CONTROL GROUP

S.No	Patient Name	Age	Gender	LDH
1.	Venugopal	70	Male	222
2.	Kandanvel	60	Male	398
3.	Rathinam	50	Female	276
4.	Subramaniam	60	Male	210
5.	Senthamani	33	Female	432
6.	Prema	58	Female	202
7.	Sampoornam	45	Female	181
8.	Shanmuga Sundaram	46	Male	309
9.	Muneshwari	47	Female	274
10.	Mallliga	58	Female	220
11.	Senthamarai	48	Female	190
12.	Kailasaam	47	Male	383
13.	R V Mani	43	Male	596
14.	Rosiyan	51	Male	657
15.	Shanmugam	52	Male	446
16.	Sadaiyappan	60	Male	520
17.	Divya	26	Female	353
18.	Edwina	25	Female	286
19.	Ezhilarasi	24	Female	560
20.	Chitra	30	Female	430
21.	Murugesan	42	Male	548
22.	Deivapriya	25	Female	317
23.	Perumal	45	Male	460
24.	Balasubramaniam	52	Male	540
25.	Saraswathi	60	Female	513
26.	Varadhan	50	Male	370
27.	Rajeshwari	58	Female	380
28.	Madasamy	52	Female	380
29.	Ravichandran	29	Male	360
30.	Rajavel	42	Male	390

INSTITUTIONAL ETHICS COMMITTEE
VIVEKANANDHA DENTAL COLLEGE FOR WOMEN

SPONSORED BY : ANGAMMAL EDUCATIONAL TRUST

Ethics Committee Registration No. ECR/784/Inv/TN/2015 issued under Rule 122 DD of the Drugs & Cometics Rule 1945

J. Baby John	Chair Person	Dr. (Capt.) S. Gokulanathan	Member Secretary
K. Jayaraman	Social Scientist	Mr. A. Thirumoorthy	Legal Consultant
R. Jagan Mohan	Clinician	Dr. N. Meenakshiammal	Medical Scientist
B.T. Suresh	Scientific Member	Dr. R. Natarajan	Scientific Member
Sachu Philip	Scientific Member	Mr. Kamaraj	Lay Person

No: VDCW/IEC/ 17/2015

Date: 14.12.2015

TO WHOMSOEVER IT MAY CONCERN

Principal Investigator: Dr Edwina. J

Title: Evaluation of Salivary Biomarker- lactate dehydrogenase in tobacco users, oral leukoplakia and oral squamous cell carcinoma – a comparative study.

Institutional ethics committee thank you for your submission for approval of above proposal. It has been taken for discussion in the meeting held on 08 .12.15.The committee approves the project and it has no objection on the study being carried out in Vivekanandha Dental College For Women.

You are requested to submit the final report on completion of project.Any case of adverse reaction should be informed to the institutional ethics committee and action will be taken thereafter.

CHAIRMAN
INSTITUTIONAL ETHICS COMMITTEE
VIVEKANANDHA
DENTAL COLLEGE FOR WOMEN
Elayampalayam-637 205
Tiruchengode (Tk) Namakkal (Dt),
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